
STUDIES ON HUMAN OVARIAN TUMOURS

by

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I N T R O D U C T I O N

These studies on ovarian tumours were largely based on the collection of gynaecological pathology material in the Department of Obstetrics and Gynaecology of the University of Edinburgh. All ovarian tumour cases examined during a period of 10 years, 1954-1963 were reviewed. These amounted to a total of 1734 cases. For the purpose of some studies, the review was extended beyond the 10 year period.

The studies included in this thesis fall into three parts. In part I, biostatistical and general aspects of the ovarian tumour series are presented. Part II deals with histopathological studies. In part III, the application of cytogenetic techniques to the study of some problems of ovarian tumours is presented and discussed.

P A R T I

BIostatistical AND GENERAL ASPECTS

PART I

BIostatistical AND GENERAL ASPECTS

INCIDENCE

It is difficult to arrive at exact figures to describe the incidence of ovarian tumours in the general population. However, an idea about their relative incidence may be obtained from review of the routine gynaecological pathology material. During a period of 10 years, 1954 - 1963, the total number of cases received in the pathology laboratory of the Department of Obstetrics and Gynaecology of the University of Edinburgh was 63,938. Of these, 1734 were cases of ovarian tumours, a relative incidence of 2.7 per cent.

The general incidence of ovarian tumours, expressed as a percentage of the total number of admissions in a gynaecologic clinic, varied widely in various reports from 1.4 to 9.9 per cent (Selye 1946). This is certainly due to the different standards according to which patients are admitted in the various clinics. Obviously, the incidence of ovarian tumours will be comparatively low in those hospitals in which women are admitted for minor ailments requiring no operative intervention. Based on a collected series from the literature of 160,324 cases of hospital admissions, Selye (1946) calculated the incidence of ovarian tumours to be 2.8 per cent in the average gynaecological clinic. This figure is remarkably similar to our own figure for the frequency of ovarian tumours in gynaecological pathology material.

AGE DISTRIBUTION

The age of the patients in our series of ovarian tumours ranged from 10 to 92 years. Table I shows the age distribution in 1721 patients with benign and malignant ovarian tumours. In the remaining 13 cases in the series, the age of the patients was not stated.

Table I

Age distribution in patients with ovarian tumours

	range	-20	-30	-40	-50	-60	60+	total
Benign	10 - 92	29	197	213	264	235	303	1241
Malignant	14 - 85	7	14	34	95	150	180	480
Total	10 - 92	36	211	247	359	385	483	1721

The rising number of both the benign and malignant ovarian tumours with age can be noted. This is also clearly demonstrated in the accompanying graph (Figure I). Ovarian tumours can occur, though very rarely, in patients below the age of 10 years. However, these are usually dealt with by the pediatric surgeon and not the gynaecologist.

Comparison of the age distribution of benign and malignant ovarian tumours can also be seen in Figure I. In the case of benign tumours, after a steep rise in the number of cases in the 3rd decade, the increase in the number of cases with age is not marked. In the case of the malignant tumours, on the other hand, the increase in the number of cases with age is more marked and a steep rise occurs in the 5th decade. It is also interesting to note that the 7 malignant tumours below the age of 20 years were all of rare type: 1 malignant teratoma, 1 disgerminoma, 2 mesoblastomas and 3 arrhenoblastomas. The youngest patient with the common ovarian adenocarcinoma in this series was 22 years old and the tumour was a mucinous adenocarcinoma.

From a clinical point of view, it is interesting to correlate the frequency of malignancy in ovarian tumours with the age of the patients. Figure 2 shows the percentage of malignant tumours among the total number of ovarian tumours in each decade. In spite of the

higher age incidence of malignant tumours, the frequency of the benign tumours is such that at no age period was the chance of an ovarian tumour to be malignant more than or equal to its chance of being benign. The percentage of malignant tumours was quite high below the age of 20 years (due to the rarity of benign tumours, not to the frequency of malignant tumours). The percentage fell in the next two decades (due to the frequency of benign tumours and also the relative rarity of malignant tumours). After the 4th decade, the percentage of malignant tumours rose again (due to the rather steady number of benign tumours and the rapidly increasing number of malignant tumours). Application of the χ^2 test to the figures in Table 1 shows that the differences in age distribution between the benign and malignant cases is statistically significant ($p \leq 0.01$)

BILATERALITY IN OVARIAN TUMOURS

Ovarian tumours show a decided tendency to involve both ovaries. This applies not only to malignant tumours but to benign tumours as well. The implications of this observation are threefold: pathogenetic, diagnostic and surgical. The pathogenetic implication in a bilateral tumour is that a general rather than an isolated local factor is probably underlying the development of the tumour. The diagnostic importance stems from the difference in the tendency to bilaterality among the various types of ovarian tumours and especially between benign and malignant tumours. From the surgical point of view, this tendency to bilaterality is an argument for the excision of the contra-lateral grossly normal ovary.

In attempting to assess the clinical diagnostic value of bilaterality, we excluded from the study tumours encountered in grossly

normal ovaries. Thus, we were left with 1649 tumours. Of these, 195 were bilateral, an incidence of about 12 per cent. The distribution of these cases is shown in Tables II and III. Although primary malignant tumours were nearly three times as commonly bilateral as benign tumours, the chance of a primary bilateral tumour being benign was about as equal to its chance of being malignant. This is due to the more relative frequency of benign tumours.

Table II

Bilaterality in ovarian tumours

	Bilateral	Total
Adenocarcinoma	85	357
Serous cystadenoma	31	292
Secondary	23	60
Benign cystic teratoma	21	239
Mucinous cystadenoma	19	464
Cystadenofibroma	8	35
Fibroma	6	106
Brenner tumour	1	16
Thecoma	1	8
Others	-	72
TOTAL	195	1649

Note: Tumours detected in clinically normal ovaries are not included.

Table III

Bilaterality in benign and malignant ovarian tumours

	Benign	Malignant primary secondary		Total
Unilateral	1091	326	37	1454
Bilateral	87 (8%)	85 (26.0%)	23 (62%)	195 (13%)
TOTAL	1178	471		1649

The relation of tumour bilaterality to the age of the patients

The incidence of bilaterality in ovarian tumours, benign and malignant, in the various age groups is shown in Table IV. The incidence of bilaterality, in general, is nearly doubled after the age of 40 years. In the benign tumours, however, an increase in incidence of bilaterality was marked only after the 6th decade.

Table IV

Bilaterality in ovarian tumours in the different age groups

		-30	-40	-50	-60	60+	Total
Benign	bilateral	13 (5%)	12 (6%)	16 (7%)	14 (6%)	32 (11%)	87 (8%)
	total	224	209	235	220	279	1167
Malignant	bilateral	2 (10%)	6 (18%)	31 (37%)	37 (24%)	32 (18%)	108 (24%)
	total	21	34	84	146	174	459
Total	bilateral	15 (6%)	18 (7%)	47 (15%)	51 (14%)	64 (14%)	195 (12%)
	total	245	243	319	366	453	1626

The ratio of malignant tumours in the bilateral ovarian tumours in the various age groups is shown in Figure 3. A bilateral ovarian tumour was more commonly malignant in the 4th and 5th decade only. Earlier, bilateral tumours were more commonly benign. Later, the chance was almost equal. Application of the χ^2 test to the figures in this analysis shows that the differences between benign and malignant tumours in the aspect of bilaterality were statistically significant ($p \leq 0.01$).

Heterogenous bilaterality

It should be remembered, in the case of bilateral tumours of the ovary, that the tumours may be of different types. We encountered several examples of these associated tumours. A benign cystic teratoma on one side with a mucinous cystadenoma on the opposite side was encountered in 3 cases. A benign serous cystadenoma on one side with a malignant serous cystadenocarcinoma in the opposite side was also encountered in several cases. The simultaneous occurrence of two primary ovarian adenocarcinomas of different types in both ovaries is more difficult to prove. In two cases in this series, the presence of a well differentiated adenocarcinoma in one ovary and a completely undifferentiated adenocarcinoma in the opposite ovary suggested this possibility. However, the possibility of a metastatic origin for the undifferentiated tumour cannot be definitely excluded. We have also encountered two examples of a mesenchymoma in one ovary and a primary adenocarcinoma in the opposite ovary.

FREQUENCY OF THE VARIOUS TYPES OF OVARIAN TUMOURS

It is not the intention here to add another elaborate classification to the already numerous ones for ovarian tumours. The types of ovarian tumours in this consecutive series of 1734 cases, are arranged in the order of their frequency in Table V. According to their relative frequency, ovarian tumours may be placed into 4 groups: common, uncommon, rare and very rare tumours.

1. Common ovarian tumours: This group includes the common benign neoplastic cysts (the mucinous cystadenoma, the serous cystadenoma and the benign cystic teratoma) and the ovarian adenocarcinoma. Each of these types accounts for more than 10 per cent of all ovarian tumours. Grouped together, these types accounted for 78 per cent of all the ovarian tumours in our series.
2. Uncommon ovarian tumours: This group includes the mesenchymal ovarian tumours (granulosa cell tumour, thecoma, Brenner tumour, adenofibroma and fibroma), the struma ovarii and secondary ovarian tumours (excluding the Krükenberg tumour). Each of the tumour types included accounts for 1-10 per cent of all ovarian tumours. Grouped together, these tumours accounted for 20 per cent of all ovarian tumours in our series.
3. Rare ovarian tumours: The tumour types included under this heading are the dysgerminoma, arrhenoblastoma, lipoid cell tumour, Krükenberg tumour and malignant transformation in benign cystic teratoma. Each of these tumours accounts for less than 1 per cent but more than 1 in 1000 of the total ovarian tumours. Grouped together, these tumours accounted for 1.5 per cent of all ovarian tumours in our series.

4. Very rare ovarian tumours: Under this heading are included the rarest types of ovarian tumours. In this group, our series included 2 mesodermal mixed tumours, 2 mesoblastomas (Teratomas), a malignant Brenner tumour, a malignant adenofibroma and a solid malignant teratoma. This group accounted for less than 0.5 per cent of the total number of ovarian tumours in our series.

To put the relative frequency of these groups of ovarian tumours in a rough and approximate way -

Ten ovarian tumours are needed to get examples of all the types of the common ovarian tumours.

One hundred ovarian tumours are needed to get examples of all the types of the uncommon ovarian tumours.

One thousand ovarian tumours are needed to get examples of all the types of the rare ovarian tumours.

More than one thousand ovarian tumours are needed to get examples of all the types of the very rare ovarian tumours.

A brief comment on the various types of ovarian tumours will be given. This will also include an orientation to studies which will follow in other chapters.

Table V

TYPES OF OVARIAN TUMOURS IN A CONSECUTIVE SERIES OF 1734 CASES

	Number of cases	
Common:		1352(78%)
Mucinous cystadenoma	464	
Primary adenocarcinoma	357	
Serous cystadenoma	292	
Benign cystic teratoma	239	
Uncommon:		349(20%)
Fibroma	135	
Secondary	54	
Adenofibroma and cystadenofibroma	50	
Granulosa cell tumour	41	
Brenner tumour	34	
Struma ovarii	18	
Theca cell tumour	17	
Rare:		26(1.5%)
Krükenberg tumour	10	
Malignant transformation in benign cystic teratoma	5	
Arrhenoblastoma	5	
Disgerminoma	3	
Lipoid cell tumour	3	
Very Rare:		7(0.5%)
Mesodermal mixed tumour	2	
Mesoblastoma	2	
Malignant Brenner tumour	1	
Malignant adenofibroma	1	
Malignant teratoma	1	
TOTAL	<hr/> 1734	

COMMON EPITHELIAL TUMOURS OF THE OVARY

This group includes the most frequent types of ovarian tumours. In our series, 1113 out of the total of 1734 tumours were in this group.

Recently the cancer committee of the International Federation of Gynaecology and Obstetrics has set out to standardize the classification of malignant ovarian neoplasms so that therapeutic reports throughout the world may be more accurately compared. For this purpose, a conference was convened at the Radiumhemmet in Stockholm in August 1961. The conference, whose members were distinguished gynaecological pathologists, considered in detail only the classification of the common epithelial tumours of the ovary, those generally considered to arise from the ovarian peritoneal epithelium. The histological classification recommended by the conference was subsequently adopted by the General Assembly of the International Federation of Gynaecology and Obstetrics, at a meeting in Mar del Plata, in September 1964. This classification was as follows:

Histologic classification of the common primary epithelial tumours of the ovary

1. Serous cystomas:

- a. Serous benign cystadenomas
- b. Serous cystadenomas with proliferative activity of the epithelial cells and nuclear abnormalities but with no infiltrative destructive growth (low potential malignancy).
- c. Serous cystadenocarcinomas

2. Mucinous cystomas:

- a. Mucinous benign cystadenomas
 - b. Mucinous cystadenomas with proliferative activity of the epithelial cells and nuclear abnormalities but with no infiltrative destructive growth (low potential malignancy).
 - c. Mucinous cystadenocarcinomas.
3. Endometrioid tumours (similar to adenocarcinomas in the endometrium):
- a. Endometrioid benign cysts
 - b. Endometrioid tumours with proliferative activity of the epithelial cells and nuclear abnormalities but with no infiltrative destructive growth (low potential malignancy)
 - c. Endometrioid adenocarcinomas.
4. Concomitant carcinoma, unclassified carcinoma (tumours which cannot be allotted to one of the groups 1, 2 or 3).

(Examples of these tumour types are shown in figures 4 - 12)

The two main features in this classification are the introduction of a borderline group between frankly benign and frankly malignant cases and the introduction of the endometrioid group of tumours.

The introduction of a borderline group in this classification is of much help. Malignancy is to be considered as a quality variably present, rather than as an entity (Taylor 1959). No valid comparison between different results of treatment can be made without segregation of this group of borderline cases. A warning, however, must be stated. This group should not be used too frequently to avoid deciding either way. A pathologist is safe with a borderline diagnosis! It is important to remember that examination of other areas of the tumour in these cases may well push a borderline case into the definitely malignant class. The diagnosis of a borderline

or potentially malignant lesion should only be made after a careful and thorough examination of several representative areas of the tumour.

The introduction of the endometrioid group meets the realization of pathologists that many cases of ovarian carcinoma show a histological similarity to endometrial carcinomas (Kottmeier 1952, Santesson 1961, Long and Taylor 1964). Whether benign endometrial lesions in the ovary should be classified as neoplasms, as suggested in the classification, is, however, debatable. The relationship of endometrioid carcinoma of the ovary to benign endometriosis raises the question of the possibility and frequency of malignant transformation in ovarian endometriosis. A special study of this problem will be presented in another part of this thesis.

It should also be noted that mixed pictures do occasionally occur in these epithelial tumours. A serous cystadenoma may show mucinous areas (Figure 13). It is not uncommon for an ovarian carcinoma to show serous, mucinous as well as endometrioid areas (Figures 14, 15). The predominant pattern should be taken as the type of the tumour in these cases.

Completely undifferentiated ovarian carcinomas are rare. Examination of other parts of the tumour often shows other more differentiated areas (Figures 16, 17). It is mostly in cases where only a biopsy of the tumour is available for examination that difficulty in classifying the tumour may not be overcome.

We have reclassified our tumours according to the FIGO classification. The result is shown in Table VI.

Table VI

Classification of the common epithelial tumours of the ovary

	Serous	Mucinous	Endometrioid	Unclassified	Total
Benign	292	464	(592)	-	756
Borderline	24	14	2	-	40
Malignant	223	31	48	15	317
TOTAL	539	509	50	15	1113

Attention may be drawn to the relatively small number of cases in the borderline and the unclassified groups. Careful examination will commonly help to place these cases in other appropriate groups. Due to their potential of malignancy, we have included the borderline cases in the group of ovarian adenocarcinoma in the general tabulation of ovarian tumours.

Analysis of our figures suggests that the three groups of serous, mucinous and endometrioid tumours (including endometrioid benign cysts) occur with about equal frequency. If it is accepted that all the three types generally arise from the surface epithelium of the ovary, which retains its capacity to differentiate along Müllerian lines, then it is probable that this differentiation proceeds equally to a tubal, endometrial or cervical pattern. The potential of malignancy, however, differs markedly in the three groups. Most of the mucinous and endometrioid growths are benign whereas almost half the serous tumours are malignant.

OVARIAN TERATOMA

The following classification is suggested for the cases of ovarian teratoma. It is modified from the classification outlined by Peterson (1957).

1. "Benign cystic teratoma": This is a benign cyst containing derivatives of two or three germ layers with differentiation and maturity of tissue elements.
2. "Malignant transformation in a benign cystic teratoma": In this type anaplastic changes are confined to a specific tissue element or portion of that element, with the remaining tissue in the tumour continuing to exhibit benign properties. This type should be sharply distinguished from the next type, the solid malignant teratoma.
3. "Malignant teratoma": This term should be restricted to the solid tumour composed of derivatives of all three germ layers. Its tissue elements show a varying degree of immaturity and lack of differentiation. The so-called "solid histologically benign teratoma" should be considered as a well-differentiated solid malignant teratoma.
4. "Teratoma with one-sided development": The well-known example of tumours in this group is the teratomatous Struma Ovarii. Other examples are debatable.

The frequency of the four types of the ovarian teratoma may be described according to our previously outlined tabulation as follows. The benign cystic teratoma is one of the common tumours of the ovary. The struma ovarii is one of the uncommon tumours of the ovary. The benign cystic teratoma with malignant transformation is one of the rare tumours of the ovary. The malignant teratoma is one of the very rare

tumours of the ovary. The frequency of these cases in our series has already been shown in Table V.

Benign cystic teratoma:

The benign cystic teratoma accounted in our series for 14 per cent of all ovarian tumours. It also accounted for 24 per cent of all benign neoplastic cysts. Below the age of 50 years, the benign cystic teratoma accounted for about one third of all benign neoplastic cysts. Table VII compares the age distribution in the three types of benign neoplastic cysts of the ovary. The predilection of the benign cystic teratoma to the childbearing period of age can be seen.

Table VII

Age distribution of benign neoplastic cysts of the ovary

	range	-20	-30	-40	-50	-60	60+	Total
Serous cystadenoma	10-81	10	48	58	49	59	67	291
Mucinous cystadenoma	16-92	6	70	70	98	102	116	462
Benign cystic teratoma	15-76	10	69	66	62	13	13	233
TOTAL	10-92	26	187	194	209	174	196	986

The association of benign cystic teratoma of the ovary with pregnancy is relatively not infrequent. Figure 18 shows such an example, with pregnancy decidual reaction in the ovarian wall of the cyst.

Striking examples of the phenomenon of tissue correlation are often seen in the benign teratoma. The well-known appearance of

stratified squamous epithelium with sebaceous glands, hair follicles, sweat glands and adipose tissue is an example. Respiratory epithelium is often associated with salivary mucous and serous glands, cartilage and sometimes thyroid acini (Figure 19). The development of bone marrow tissue in between the trabeculae of bone may also be observed (Figure 20). The association of brain tissue with choroid plexus structure and ependyma is not infrequent (Figure 21). These tissue correlations may be looked upon as abortive attempts towards organ formation. The foetiform nature of teratomas has been the subject of controversy, in old times and also recently. A discussion of this problem in the light of the recently introduced methods of cytogenetic study will be presented in another chapter in this thesis.

Malignant transformation in a benign cystic teratoma:

Five examples of malignant transformation in a benign cystic teratoma were encountered in this study. During the same period, 239 benign cysts were encountered, and the total number of malignant ovarian tumours was 482. In the review of Peterson (1957), the overall incidence of malignant degeneration in a benign cystic teratoma was 1.8 per cent. According to the same author, the consensus of the literature places the incidence in the neighbourhood of 1.5 per cent of all ovarian malignancies. According to Matz (1961), the vast majority of the reported cases were epidermoid carcinomas (80 per cent); less commonly the tumour was an adenocarcinoma, sarcoma (gliosarcoma, osteogenic sarcoma, melanosarcoma), carcinoid, chorionepithelioma or malignant struma ovarii. Of our 5 cases, two were squamous cell carcinomas (Figure 22), two were mucinous adenocarcinomas arising in gut epithelium (Figures 23, 24)

and one was an adenocarcinoma of doubtful origin. More recently, I have encountered an example of the rare carcinoid tumour or argentaffin carcinoma (Figures 25, 26), in relation to a benign cystic teratoma.

It is worth remembering that the presence of a malignant tumour in the same ovary as a benign cystic teratoma does not necessarily mean the origin of this tumour in the tissues of the teratoma. If the origin of the tumour from the teratoma is not demonstrable, and the histological picture of the tumour is different from that of primary malignant ovarian tumours, the possibility of secondary tumours should be considered. I have recently seen an example of secondary bronchial carcinoma in the ovary, in association with a benign cystic teratoma. In another case, a disgerminoma was present in association with a benign cystic teratoma. The latter association and its possible significance will be referred to in discussing the disgerminoma.

The age incidence in our 5 cases of malignant transformation in benign cystic teratoma ranged from 48 to 63 years. According to the review of Peterson (1957), 63 per cent of reported cases were over 40 years when the disease was discovered. This high age incidence is in contrast to the commonly young age of the patients with the malignant solid teratomas and emphasizes further the distinction between the two groups.

Carcinoid tumours of the ovary are rare. In 1939, the first two recorded cases of an argentaffin carcinoma occurring in the wall of ovarian teratoma were reported by Stewart et al. (1939). Occasional cases have been reported since then. Doucette and Estes (1965) mentioned 16 reported cases of primary ovarian carcinoid tumours,

13 of which have been associated with benign cystic teratomas. The two authors presented a 14th case. The apparent teratomatous tissue of origin of the carcinoid component, when recognized, was gastro-intestinal in 6, respiratory in 5, and unspecified entodermal in 1. An interesting aspect of these rare tumours is the possibility of functioning activity leading to the well-known carcinoid syndrome. The first report of flushing and cutaneous manifestations of functioning argentaffin elements found solely in an ovarian tumour was probably that of Kierland et al. (1958). It is worth remembering functioning carcinoid tissue as another cause of vasomotor symptoms in women. The presence of a palpable ovarian tumour coincident with abnormal vaso-motor activity should arouse suspicion concerning serotonin producing structures. The utilization of the simple urinary test for 5-Hydroxy Indole Acetic Acid (5-HIAA) can be of diagnostic help. In our carcinoid tumour, there was no definite evidence that the tumour was functioning.

Solid malignant teratoma:

Malignant teratomas of the ovary are tumours composed of a mixture of tissue forms, both mesenchymal and epithelial, of a variable degree of differentiation and resembling the tissues of the embryo. According to Murray and Hofmeister (1961), 54 cases have been reported in the world literature. Bren and Neubecker (1963), reported another 17 cases. In our series, the solid malignant teratoma was one of the very rare ovarian tumours. Only one case was encountered. The teratomatous elements in this tumour were apparently mature and of adult type. However, the recurrence of the tumour proved its potential of malignancy. For its rarity and for its

interesting features, this case will be discussed in detail under the heading of very rare ovarian tumours, in another chapter of this thesis.

More recently, I have seen two other cases of solid malignant teratoma in the ovary. One was removed from a girl aged 10 years. Most of the tissues of the tumour were poorly differentiated. However, the picture was variable in different parts of the tumour. Figure 27 for example shows mature epidermal tissue. Figure 28 from another part shows an extremely anaplastic pattern, with a superficial resemblance to malignant trophoblast. Figure 29 from a third part of the tumour may suggest the picture of dysgerminoma, another possibly germ cell tumour (Bren and Neubecker 1963). The other tumour, removed from a patient aged 39 years, showed well differentiated teratomatous elements. The tumour was partly solid and partly cystic (Figure 30). The cystic part, however, was lined not by squamous epithelium but by neural tissue (Figure 31) (Unfortunately, the composition of the fluid content of this cystic part was not analysed). Figures 32-34 show tissues from the solid part of the tumour. Figure 32 shows mature skin elements. Figure 33 shows salivary glands and respiratory epithelium. Figure 34 shows nerve ganglion from the muscle wall of a gut-like structure.

Struma Ovarii

The presence of thyroid tissue in a benign cystic teratoma is not uncommon (in 19 per cent of cases according to Emge, 1940). However, the predominance of this tissue in the teratoma is uncommon. Five cases in our series showed this picture of teratomatous struma ovarii. In 13 other cases, no other teratomatous elements were seen

in the available sections from the tumour. These cases may be labelled "pure" struma ovarii, though admittedly a more thorough examination may have probably revealed other teratomatous structures in at least some of these cases.

It is not known why the thyroid tissue should occasionally overgrow other elements in the teratoma of the ovary. The patterns of age distribution do not appear to be the same in the struma ovarii and the teratoma. In our series, all the cases of benign cystic teratoma with the exception of 36 cases (out of 233) were encountered between the ages of 20 and 50 years. Of the 18 cases of struma ovarii, only 8 cases were encountered between the ages of 20 and 50 years. Nieminen et al. (1963) found the average age of their 19 cases of struma ovarii to be 12 years higher than that expected for the benign cystic teratoma. Another point that may be worthy of note is that none of our cases of struma ovarii was bilateral. This was also the experience of Nieminen et al. (1963). The tendency of the benign cystic teratoma to be bilateral is well-known.

Thyroid tissue in the ovary is liable to the same functional and pathologic changes as thyroid tissue in the neck (Novak and Woodruff 1962). In one of our cases, the structure of the thyroid tissue closely resembled that of the thyroid foetal adenoma (Figure 35).

One-sided development in the ovarian teratoma:

The predominance of one type of tissue in the benign cystic teratoma is referred to as one-sided development. The predominance of cutaneous tissue elements in the well-known dermoid cyst can actually be regarded as an example of this phenomenon. The struma ovarii is another well-known example. The solid sebaceous gland tumour of the ovary reported by Strauss and Gates (1964) is probably

another but unusual example. The very rare examples of non-gestational choriocarcinoma of the ovary are also in this category.

The origin of other tumour types in the ovary as one-sided development in teratomas is more debatable, and the concept has been rather abused to explain the obscure origin of some ovarian tumours. It is interesting to mention here only two tumour types: the mucinous cystadenoma and the mesonephroma of Schiller. It has been maintained that mucinous cystadenomas represent teratomas in which a tall, columnar entodermic epithelium has overridden and blotted away other tissues in the teratoma (Novak and Woodruff 1962). Willis (1960), on the other hand, rejects this hypothesis. We have encountered 9 examples of the association of mucinous cysts and benign cystic teratomas, of which 7 were on the same side. The tumours varied from a dermoid cyst with large mucinous locules to mucinous cystadenomas with the dermoid cyst forming a small part of the tumour. With the exception of these 9 cases, there was no suggestion, in our 464 cases, of a teratomatous origin. It may be that a teratomatous origin accounts for some of the cases of mucinous tumours in the ovary. However, in the great majority of cases, evidence for such an origin is not apparent. Demonstration of argentaffin or argyrophil cells in some of these cases has been suggested as an evidence for an enteroid origin (Fox et al. 1964).

The interpretation by Teilum of certain tumours, described as mesonephromas by Schiller (1939), as extraembryonic mesoblastomas is interesting. Teilum compares the structure of the tumour to the extraembryonic mesoderm, with the epithelial spaces interpreted as vitelline in character (Mesoblastoma vitellinum, Teilum 1965). The possibility that a tumour (apparently of teratomatous origin) may

develop only the structure of extraembryonic mesoderm is worthy of consideration. Teratomas, especially in the testis, may develop mainly the structure of extra-embryonic ectoderm or trophoblast (the chorioneplithelioma). The interpretation of the mesonephroma of Schiller will be referred to again in the chapter about the very rare tumours.

BRENNER TUMOUR

Our poor understanding of the nature of the Brenner tumour is easily evident from the fact that it is the only primary ovarian tumour that has no pathological designation, other than its eponymic title. The interesting story behind this eponymic title was revealed by Speert (1956). Dr. Fritz Brenner, who emigrated to Africa before the first world war, was no more aware in 1956 than his fellow general practitioners in Johannesburg, that his own name was inseparably attached to the tumour he described long back in 1907! Brenner's original concept was that the tumour arose from the ovarian follicular tissue: "Das oophoroma folliculare" (Brenner 1907). Meyer (1932), in his classical paper on this tumour, sharply differentiated it from the granulosa cell tumours, emphasized its lack of endocrine activity, noted its association with the mucinous cystadenoma, attributed its origin to Walthard nests and also popularized the eponymic title of Brenner tumour. Since then, several series and studies discussing this tumour and agreeing or diverging from Meyer's concept have appeared in the literature. It is of interest, at least historically, to note the recent trend of going back to Brenner's original concept. Teoh (1953), for example, states his belief that Brenner's opinion, implied in his term "Oophoroma

folliculare", that this tumour came from ovarian follicular tissue, was correct and that the histo-genesis of the Brenner tumour is the same as that of the granulosa-theca cell series of tumours and that its "typical" structure which makes it appear to be distinct from the "typical" granulosa cell tumours, is the result only of a change in form with metaplasia of the epithelial cells.

In our series of 1734 ovarian tumours, 34 Brenner tumours were encountered, an incidence of about 2 per cent. A notable feature, however, was that in 18 of the 34 cases the ovary was not or only slightly enlarged (Figs. 36, 37). In one more case, it was an accidental finding in the wall of a tuboovarian abscess. The true incidence of the Brenner tumour may possibly be more than these figures indicate. Many of the small tumours can pass undetected. Small tumours in the walls of large mucinous cystadenomas may also be missed. A Brenner tumour with marked overgrowth of the fibromatous tissue may also be reported as a simple ovarian fibroma.

The age of the patients ranged from 22 to 80 years with an average of 46 years. Only two patients were below the age of 40 years. The fact that these tumours are slowly growing and produce symptoms mainly by their bulk can partly account for the late age of clinical presentation. The age distribution in the small tumours was not significantly different from that of the large tumours and it appears that the difference between the two groups is a difference in the rate or capacity of growth and not in the duration of the tumour before discovery. Brenner tumours in association with mucinous cystadenomas also showed the same high age incidence.

Only one tumour was bilateral in our series. This striking tendency to unilaterality has also been the experience of other

authors. Idelson (1963) could gather only a total of 33 bilateral cases from the literature.

The gross appearance of the tumours varied. As already mentioned, the tumour was an incidental pathological finding in a more or less normal ovary in 18 cases. In 6 cases, the tumour was an incidental finding in the wall of a mucinous cystadenoma. In one case, the tumour was an incidental finding in the wall of a tuboovarian abscess. Thus, in only 9 cases was the tumour the presenting feature of the case. In 6 of these, the tumour was solid and fibroma-like on gross appearance while in the 3 remaining cases the tumour was partly cystic.

The sites of the small Brenner tumours in the ovary are of histogenetic significance. Figure 38 shows a small Brenner tumour in the ovarian cortex near the surface of the ovary. An origin from rete structures as suggested by Schiller (1934) is certainly not applicable for this tumour. Figure 39, on the other hand, shows a Brenner tumour in the hilum of the ovary. A diverse origin for the Brenner tumour, as suggested by Greene (1952) may explain these findings.

The malignant Brenner tumour is very rare. I made this diagnosis in only one case. This will be considered together with other very rare types of ovarian tumours in another part of this thesis.

THE ADENOFIBROMA AND CYSTADENOFIBROMA

Data about this group of tumours have been rather scanty in the literature. Scott (1942) could collect from the literature a total of 17 tumours of this distinct group (including the case of Wolfe 1927,

as the only case previously reported in the English literature) and added 14 other cases. The study of Scott emphasized that these tumours are of germinal epithelial origin with a close relationship to serous cystadenomas and papillary fibromas and he suggested that these tumours are to be classified as a special type of epithelial tumours of the ovary under the subhead of serous cystadenoma. With the reports of McNulty (1959) and Rothman and Blumenthal (1959), the total number of reported cases was brought up to 68.

In our series of ovarian tumours, 50 cases of adenofibroma and cystadenofibroma were encountered, an incidence of 2.8 per cent. It should be noted, however, that 15 of the cases were encountered in grossly normal ovaries.

The age of the patients ranged from 26 to 82 years with an average of 49.3 years. This high age incidence is in agreement with that reported by other authors. In the review of Scott (1942), 93.5 per cent of the patients were 40 years or over, and 64.5 per cent were 50 years of age or over. In the review of Rothman and Blumenthal (1959), 30 of the 43 cases were 50 years of age or over. It is interesting to compare the age distribution of the adeno and cystadenofibroma with that of the two related tumours, the serous cystadenoma and the fibroma. This is shown in Table VIII. The adenofibroma is more related in its age distribution to the fibroma. Indeed, such high age incidence is usual for all fibromatous tumours of the ovary as we have observed in the case of the Brenner tumour.

The gross appearance of the tumours varied. As already mentioned, in 15 cases the tumours were incidental findings, producing little or no ovarian enlargement (Figures 40, 41). In 13 cases, the tumour showed the gross picture of cystadenofibroma (at least one

Table VIII

Age distribution of the serous cystadenoma, adeno- and cystadenofibroma and the ovarian fibroma

	Range	Average	40 years and over	50 years and over
Serous Cystadenoma	10-82	40.9	62%	47%
Adeno and Cystadenofibroma	26-82	49.3	88%	62%
Fibroma	20-81	51	88.4%	71.5%

fourth of the tumour mass was solid). In 3 of these, the cysts were also papilliferous. In the remaining 22 cases, the tumour presented a fibroma-like appearance.

Microscopically, the tumours showed a variable quantitative relationship of epithelial elements and fibromatous tissue. The epithelial lining ranged from a flat type to a cubical or columnar, sometimes tubal, type. Less commonly, however, the epithelium showed endometrioid or mucinous areas. Calcified psammoma bodies may also be noted.

Malignancy in the ovarian adeno- and cystadenofibroma has been rarely reported. The original report of Scott (1942) included no example of clinical or microscopical malignancy but he suggested that the potentiality in this respect would appear to be as great as in the serous cystadenoma and the fibroma. Minkowitz and Cohen (1966) could collect from the literature only 9 instances of this type of malignant transformation and in only two of these was the case completely reported. They added to these one case of their own. Our own observations in this aspect lead us to make a distinction between

the potential of malignancy in the cystic part of the cystadenofibroma and the potential of malignancy in the solid fibroadenomatous part.

1. Malignant transformation in the cystadenofibroma, involving the serous cyst, takes the form of the common papillary serous cystadenocarcinoma and is usually not distinguishable from it. When the growth is fairly advanced, its relation to the benign cystadenofibroma may be lost. Figures 42 and 44 illustrate two examples in our series in which such a change was detected at an early stage. Figure 42 shows a borderline papillary lesion in benign cystadenofibroma. Figure 44 shows an early polypoid adenocarcinoma in one of the locules of a benign cystadenofibroma.
2. Malignant transformation in the solid adenofibromatous part may, on theoretical grounds, implicate either the fibromatous or the epithelial elements. Sarcomatous transformation in the ovarian adenofibroma has not to our knowledge been reported, though it is known in the analogous fibroadenoma of the breast.

In one of our cases, the stromal elements of the tumour showed evidence of active proliferation, though not amounting to the degree of sarcomatous change (Figure 43). In this tumour thecal transformation was marked in the stroma and the cystic part of the tumour showed a borderline papillary lesion (Figure 42).

Squamous cell metaplasia and proliferation in the ovarian adenofibroma have been described (Novak and Woodruff 1962). It is quite possible, however, that these cases really represent endometriomas with an inactive epithelium and a lacking stroma, especially when they are encountered in patients past the menopause. Figures 45 - 48 illustrate two interesting examples in our series. In

Figures 45 and 46 the tumour was encountered as a localized nodule in the wall of a mucinous cystadenoma. In Figures 47 and 48 the tumour was encountered as a fibromatous area in association with a serous papillary cystadenocarcinoma. These two examples may illustrate the multiple Mullerian potentialities of the surface epithelium of the ovary.

Apart from these cases of adenoacanthoma the epithelial elements of the adenofibroma may undergo a frank carcinomatous change. This change should be a very rare event. We have encountered only one case in this series. More recently, we have diagnosed another case. The histological picture is also rather distinctive, and it is this type of tumour which deserves the designation as malignant adenofibroma. In view of its extreme rarity, a more detailed description of this type of tumour will be given together with the other very rare types of ovarian tumours in another part of this thesis.

THE OVARIAN FIBROMA

The relative frequency of the ovarian fibroma is not generally realized. In our series, 135 cases were fibromas, an incidence of 7.7 per cent. Of these, however, 29 were encountered in normal-sized ovaries (Figures 49, 50).

Clinically, solid ovarian tumours are regarded as suspicious of malignancy. In our consecutive series, however, solid benign ovarian tumours were not uncommon. If we exclude tumours detected in clinically normal ovaries (as these have no diagnostic clinical significance), the total number of cases of fibroma, adenofibroma, Brenner tumour and thecoma was 165. The total number of primary malignant ovarian tumours during the same period was 412. Of these,

probably the majority were cystic (Novak and Woodruff 1962). The solid benign tumours share the same high age incidence as the malignant tumours, as we have shown in previous discussions. It may be noted, however, that bilaterality is not as common in these benign solid tumours as in the malignant tumours (Table II). Bilateral solid tumours are more commonly malignant.

GRANULOSA AND THECA CELL TUMOURS

In our material, the 58 cases of granulosa and theca cell tumours accounted for 3.2 per cent of all ovarian tumours. Forty-one of the cases were granulosa cell tumours and 17 were theca cell tumours. Included in the granulosa cell tumours, however, are cases showing prominent thecomatous tissue as well as the granulosal elements. It should also be noted that it is sometimes difficult to differentiate with certainty a granulosa cell tumour from a thecoma. These are the cases in which the granulosa cells are diffusely arranged with no definite structural pattern, follicular or trabecular. Again, it should also be mentioned that the distinction between a thecoma and a fibroma is not always sharp and perhaps a more thorough examination of cases of fibroma may disclose more tumours with thecomatous areas.

A comparison of the age distribution of the cases of granulosa cell tumours and the theca cell tumours is shown in Table IX.

The later age incidence of thecomas is apparent in this table.

With the exception of one case of bilateral theca cell tumour, all the tumours were unilateral.

Table IX

Age distribution of granulosa and theca cell tumours

	range	-29	-39	-49	-59	60+	Total
Granulosa cell tumours	27-78	2	3	8	10	18	41
Theca cell tumours	51-80	-	-	-	6	11	17

An interesting feature in this series was that in 16 of the cases, the tumour was detected after operation in a clinically normal ovary. These small tumours present several interesting features. These will be the subject of a special study in another chapter.

ARRHENOBLASTOMA

The arrhenoblastoma is one of the rare types of ovarian tumours. Only 5 cases in our series were classified as arrhenoblastomas.

It has been customary to regard the arrhenoblastoma and the granulosa cell tumours, as tumour types which are sharply distinct in their morphology, histogenesis as well as in their endocrine potential. This distinction does not seem now to be so exact. As regards the endocrine potential, it has been suggested that both types contain feminizing and masculinizing cells and so are potentially bisexual. Thus, Teilum (1949) recognizes the Sertoli cell as feminizing and the Leydig cell as masculinizing. Shippel (1955) recognizes the granulosa cell as feminizing and the theca cell as masculinizing. As regards the histological distinction, most gynaecological pathologists would admit that this is not easy in many of the cases. Haines and Taylor (1962) recommend the term gonadal

stromal tumour for the type of case in which differentiation is not possible. Novak and Long (1965), after reviewing about 100 arrhenoblastomas from the ovarian tumour registry, have adopted a more defeatist attitude and recommended the term gonadal stromal tumour for all tumours of both groups, since morphological differentiation seems futile. The old distinction in histogenesis also does not seem to hold, since tumours showing mixed patterns are more frequent than what was originally recognized. Mackinlay (1957) described the presence of male cells in granulosa-theca cell tumours and recommended the deletion of the term arrhenoblastoma. Warner et al. (1960), on the other hand, recommended the generic title of gynandroblastoma for both tumour types.

We have seen examples of tumours of apparently granulosa cell type which show in areas a picture comparable to that of the arrhenoblastoma (Figures 51 and 52). We have also seen examples of tumours in which neither the histological picture nor the endocrine potential could decide whether the tumour was a granulosa cell tumour or an arrhenoblastoma and the diagnosis was really anybody's guess (Figures 53, 54). Granulosa cell tumours, however, may show a distinctive folliculoid pattern that is not present in an arrhenoblastoma. It may be concluded that the arrhenoblastoma and the granulosa cell tumour are better regarded as examples of divergent differentiation of the tumour of the gonadal stroma, rather than as separate tumour entities.

Attempts to correlate the histological structure with the endocrine potential are certainly futile. It is not necessary for an ovarian tumour to reproduce the structure of the testis in order to secrete androgens. In fact, it is not the testicular parenchyma

but the interstitial tissue which is responsible for hormone production. The "masculinizing" arrhenoblastomas really hardly resemble the testicular structure (Figure 55). It was only by a stretch of imagination and by including cases of testicular tubular adenoma that Robert Meyer could elaborate his hypothesis (Meyer, 1931b). The status of the testicular tubular adenoma as an ovarian tumour has been recently questioned in the light of recent knowledge of intersex states. The presence of testicular-like tissue in the gonad may have as its basis an underlying intersex state and not abnormal tumour differentiation of the ovary. These cytogenetic aspects of the arrhenoblastoma will be dealt with in further detail in another part of this thesis.

DISGERMINOMA

The disgerminoma is one of the rare ovarian tumours. Only 3 cases are included in this series.

One of the cases showed abnormally high gonadotrophin titres. This case has been reported in full by Hobson and Baird (1966). In another case, the tumour was encountered in association with a benign cystic teratoma in the same ovary. This association is rare, as evidenced by the review of Mueller et al. (1950) who found only 3 examples of the association in 427 cases of disgerminoma in the literature. In the testis, the coexistence of seminoma and teratoma is not uncommon. Willis (1960) believes that the association cannot be regarded as fortuitous in the testis but does not believe in a common origin for the two tumours and he suggests that maldevelopment of the testis predisposes to both neoplasms. Other authors consider such associations as an evidence for the germ cell

origin of both tumour types (Bren and Neubecker 1963).

The relationship of the dysgerminoma to intersex states will be discussed fully in another chapter of this thesis.

LIPOID CELL TUMOURS

The generic title, lipoid cell tumours, is used to refer to certain types of ovarian tumours with a high lipoid content (Haines and Taylor, 1962). These tumours are generally composed of large, rounded or polyhedral cells that resemble Leydig, lutein and adreno-cortical cells. It is doubtful, however, whether a histological distinction can be made with any certainty between these types of cells. It is also doubtful whether adrenal rests give rise to ovarian tumours (Hughesdon, 1966).

Lipoid cell tumours of the ovary are rare. Hughesdon (1966) could collect from the literature only 109 cases. We have encountered 3 examples of these tumours in our series. We interpreted two of these as hilus cell tumours and the third as a luteinized thecoma. It is interesting to note that our two cases of hilus cell tumours presented with postmenopausal bleeding and endometrial hyperplasia and showed no evidence of masculinization (Figures 56-59). Novak and Mattingly (1960) observed that a hyperplastic endometrial pattern was present in all the cases of hilus cell tumours included in their review, whenever the endometrium had been available for examination. In the third example of lipoid cell tumours in our series, the tumour was encountered in the ovarian cortex as a small nodule, detected only when the ovary was sectioned after operation (Figures 60-62). The endometrium showed postmenopausal atrophy in this case. These 3 cases illustrate the lack

of correlation between the histological pattern and the endocrine potential in these types of ovarian tumours.

SECONDARY OVARIAN TUMOURS

In clinical material, secondary ovarian tumours are uncommon. In fact the cases that are liable to be encountered fall mostly in one of the following two groups:

1. Cases of genital malignancy in which the ovaries are removed as part of the operation. Cases of breast carcinoma with oophorectomy carried out as a line of treatment also fall in this group.
2. Secondary tumours presenting as ovarian tumours with the primary lesion not evident clinically. In these cases, the nature of the tumour may be revealed during the operation or more commonly after pathological examination. This group includes, among others, the famous Krükenberg tumours. Rarely, cases of carcinoma of the body of the uterus may present with large ovarian tumours when the primary endometrial lesion is still small and localized. In these cases, however, it may be difficult to decide whether the ovarian tumour is primary or secondary. Primary malignant ovarian tumours of endometrioid character are not uncommon. In 4 of our 41 cases of ovarian secondary endometrial carcinoma, the endometrial growth was early and with no definite evidence or with only minimal evidence of invasion. In one of these 4 cases, the adenocarcinoma was present in the curettings only and the removed uterus showed no further evidence of the tumour.

The distribution of the cases of secondary ovarian tumours in our series was as follows:

Tumours secondary to a primary tumour in the genital tract:		48 cases
(endometrial carcinoma	41 cases	
fibroid sarcoma	3 cases	
cervical carcinoma	4 cases)	
Tumours secondary to an extragenital primary (typical):		6 cases
(breast	3 cases	
others	3 cases)	
Krükenberg tumours:		10 cases
TOTAL		<u>64 cases</u>

SUMMARY

A Biostatistical and General Survey of a consecutive series of 1734 ovarian tumours is presented.

1. The incidence of ovarian tumours in routine gynaecological pathology material was 2.7 per cent.
2. The age of the patients ranged from 10 to 92 years. The number of cases increased with age. The behaviour of benign and malignant tumours was, however, different. After a steep rise in the number of cases in the third decade, the increase in the number of benign tumours with age was not marked. In the case of malignant tumours, on the other hand, the increase in the number of cases with age was more marked and a steep rise occurred in the 5th decade. The percentage of malignant tumours was relatively high below the age of 20 years (due to the rarity of benign tumours, not to the frequency of malignant tumours). The percentage fell in the next two decades (due to the frequency of benign tumours and also the relative rarity of malignant tumours). After the 4th decade, the percentage of malignant tumours rose again (due to the rather steady number of benign tumours and the rapidly increasing number of malignant tumours).
3. The overall incidence of bilaterality in our series was 12 per cent. Although primary malignant tumours were nearly three times as commonly bilateral as benign tumours, the chance of a primary bilateral tumour being benign was about as equal to its chance of being malignant, due to the much more relative frequency of benign tumours. The incidence of bilaterality in ovarian tumours was nearly doubled after the age of 40 years. In benign tumours, the increase in incidence of bilaterality was marked only after the

sixth decade. A bilateral ovarian tumour was more commonly malignant in the 4th and 5th decades only. Earlier, bilateral tumours were more commonly benign. Later, the chance was almost equal. Cases of heterogenous bilaterality were also encountered.

4. According to their relative frequency, ovarian tumours were tabulated into four groups: common, uncommon, rare and very rare tumours.
 5. General observations on the various types of ovarian tumours were presented and discussed.
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P A R T I I

HISTOPATHOLOGICAL STUDIES

CHAPTER 1THE ORIGIN OF MALIGNANT OVARIAN TUMOURS IN ENDOMETRIOSIS

It is now 40 years since Sampson (1924, 1925) first pointed out the possibility of malignant transformation in ovarian endometriosis. However, its frequency and clinical and pathological characteristics remain widely unrecognised. Few studies have been made on this problem and most of the literature on the subject has been limited to the reporting of isolated cases.

Recent interest in the problem has been aroused by the more general realization among pathologists of the frequency with which endometrial-like adenocarcinoma is encountered among cases of primary ovarian carcinoma (Long and Taylor, 1964). The introduction of a new group, termed "endometrioid", into the histological classification of the common primary epithelial tumours of the ovary has already been discussed in the previous chapter. The frequency of tumours so designated was considered to warrant the status of a separate group.

As Sampson (1925) rightly pointed out, there are two methods for attacking the problem of the development of malignant transformation in ovarian endometriosis: either to study cases of benign endometriosis in an endeavour to find early malignant changes in them or to study cases of ovarian carcinoma to ascertain whether or not the carcinoma could have arisen in misplaced endometrial tissue. In our study of this problem, we decided to follow both lines of approach.

Material

This study is based on the histological material in the

laboratory during a period of 10 years, 1954 - 1963. Apart from a critical examination of the 418 primary malignant tumours encountered during this period for evidence of a possible or definite origin in endometriosis, we also reviewed all the cases of ovarian endometriosis during the same period. Among the total of 62,938 cases reported on during this period, there were 637 cases of ovarian endometriosis. In 45 of these, however, the diagnosis was only presumptive, due to the absence of well-defined endometrial elements. In the remaining 592 cases the slides were reviewed for evidence of pre-malignant or early malignant changes.

Results

Ovarian endometriosis

Atypical epithelium was encountered in four endometrial cysts, but without definite evidence of malignancy with stromal invasion. Heaping up in layers, cellular pleomorphism, variations in the size of the nuclei, nuclear hyperchromatism and mitotic figures were noted to variable degrees in these cases (Figure 63). The ages of the four patients were 45, 48, 55 and 66 years and two of them were six and ten years postmenopausal.

Another interesting observation was the presence of postmenopausal benign hyperplasia in two cases of ovarian endometriosis associated with the presence of a granulosa-theca cell tumour in the opposite ovary. The two patients were 12 and 15 years postmenopausal.

The association of endometriosis with an ovarian tumour in the same and/or the opposite ovary was observed in 52 cases. In 21 of these, the tumour was malignant and of these, seven cases showed both the endometriosis and the malignant tumour in the same ovary. The

malignant tumours in these seven cases were two adenoacanthomas, four endometrioid adenocarcinomas and one mesodermal mixed tumour. Definite evidence for the origin of the tumour in endometriosis could be established for the two adenoacanthomas and for two endometrioid carcinomas. These cases will be considered further in the next section.

Primary malignant ovarian tumours

In the survey of 418 primary malignant ovarian tumours, we classified 52 cases as having a histological appearance similar to that of malignant tumours of the uterine endometrium. Of these, 12 were adenoacanthomas, 38 were adenocarcinomas and two were mesodermal mixed tumours. These malignant "endometrioid" tumours thus accounted for about 12 per cent of all primary malignant tumours of the ovary in our series. However, unfortunately the uterus was not available for examination in 17 of these cases - three adenoacanthomas and 14 adenocarcinomas. In these cases, the possibility of a metastatic spread from a primary uterine tumour cannot be excluded on histological review.

Adenoacanthoma:

Of the 12 cases, two were shown to develop in endometrial cysts. Endometriosis was present in the opposite ovary in two other cases but could not be demonstrated in the same ovary. In four cases, the opposite ovary was not available for examination. In two instances, the adenoacanthoma was encountered as an accidental pathological finding. The first was encountered as a localized nodule in the wall of a benign mucinous cystadenoma (Figs. 45, 46). An additional interesting feature in this case was the presence of a few areas of

squamous metaplasia in the benign proliferative uterine endometrium (Figure 74). The second case occurred as a fibrous area in the wall of a serous papillary cystadenocarcinoma (Figures 47, 48). In both cases, no definite evidence of benign endometriosis with endometrial stroma could be demonstrated, and the cases were classified as adenoacanthoma developing in adenofibroma. A brief report of the two cases of definite malignant transformation in endometrial cysts will now be given.

Case reports

Case 1: (Serial number E 913, F 990) Mrs. H.D., aged 51 years, nulliparous and two years postmenopausal, was operated upon with the diagnosis of uterine fibromyomata and ovarian endometriosis. A total hysterectomy with bilateral salpingo-oophorectomy was performed. Eighteen months later the patient returned with abdominal pain, a lump and urinary frequency. Laparotomy was carried out and a bilocular completely encapsulated cyst about 10 cm. in diameter was removed from the site of the right broad ligament. During removal, one loculus ruptured with escape of chocolate brown fluid. Review of the previous sections from the ovary one and a half years before revealed the presence of an early endometrial carcinoma in an endometrial cyst (Figures 64, 65). Gross pathological examination of the second specimen showed the presence of intracystic papillae and suggested the appearance of an endometrial cyst. Sections from the tumour, on review, were diagnosed as showing adenoacanthoma. An interesting feature was the apparent malignant nature of both the glandular and squamous elements in this tumour (Figure 67). No definitely benign endometrial tissue was encountered in the sections

available from this second tumour, but the cyst was partly lined by a broad zone of pseudo-xanthoma cells (Figure 66) and old haemorrhages were abundant in the stroma. One may be inclined, however, to interpret the second tumour in this case as representing malignant transformation in an endometriotic lesion left from the previous operation, rather than a recurrence of the first localized growth.

Case 2: (serial No. F 9700) Mrs. W.B., aged 47 years, was operated upon for an asymptomatic abdominal mass. An adherent right ovarian cyst was found as well as an anterior wall fibroid. The cyst ruptured during removal with the escape of brownish stained fluid and pieces of friable tissue. Subtotal hysterectomy with bilateral salpingo-oophorectomy was carried out and the patient received a post-operative course of deep X-ray therapy. Three years later, when the patient was last seen, there was no evidence of any recurrence. Pathological examination of the cyst showed a localized papillary adenoacanthoma (Figure 68). The rest of the cyst wall showed some atrophic endometrial glands but the lining epithelium was largely replaced by a broad zone of pseudo-xanthoma cells. The other ovary contained a small blood cyst.

Endometrioid adenocarcinoma:

The histopathological characteristic of endometrioid carcinoma of the ovary, as distinct from the more common serous cystadenocarcinoma, have been outlined by Long and Taylor (1964) as follows:

1. Glandular type of cellular arrangement.
2. A more blunt appearance of papillary extensions. The papillary serous cystadenocarcinoma is characterized by fine branching papillae.

3. The border of the multi-layered endometrial growth has a less uneven appearance than that seen in the serous type in which the margin projects in a more papillary pattern.
4. Presence of squamous metaplasia or acanthosis.

Evidence of benign endometriosis was demonstrable in the same ovary in four of our cases of endometrioid carcinoma. In two of these, the carcinoma could be clearly shown to be arising from the endometriosis. In the other two cases, the relation was highly suggestive but not definite. Endometriosis was encountered in the opposite ovary but not on the side of the tumour in four other cases. In one of these, the endometriosis showed pronounced cystic hyperplasia. The opposite ovary was available for examination in 15 cases. A brief report of the two cases of definite malignant transformation in endometrial cysts will now be given.

Case reports

Case 3: (serial no. F 2839) Miss J.L., aged 46 years, presented with acute abdominal pain and a tender pelvi-abdominal mass. Laparotomy showed that the peritoneal cavity was filled with a light brown opaque fluid, having its source from a ruptured left ovarian cyst. The cyst was thick walled, adherent and still distended to about the size of a grapefruit by some brown thick fluid. Pathological examination of the removed cyst showed that it was an endometrial cyst with areas of endometrial cystic hyperplasia and areas of papillary adenocarcinoma (Figure 69).

Case 4: (serial no. G 2364) Miss B.S., aged 37 years, presented with recurrent attacks of hypogastric pain and an enlarging abdominal lump. A left ovarian cyst, together with the uterus and

the right ovary were removed. The cyst was about 15 cm. in diameter and contained copious amounts of dark fluid. Multiple small papillomata were seen to fill almost half the cyst cavity. Microscopical study showed a papillary endometrioid adenocarcinoma arising into and continuous with the benign epithelial lining of an endometrial cyst (Figures 70, 71). The opposite ovary was the seat of endometriosis.

Mesodermal mixed tumours

Primary mesodermal mixed tumours of the ovary are extremely rare. Two cases in our series were so diagnosed, on re-evaluation of the pathological sections of the tumours. The fact that these tumours are usually uterine justifies their classification as one type of endometrioid tumours of the ovary. A detailed report of the two cases will be given in another chapter, under the heading of very rare ovarian tumours. It may be mentioned here, however, that in one of the two cases one part of the tumour showed the picture of a benign adenomyoma. Unfortunately, however, in the absence of the original specimen, we could not demonstrate to our satisfaction a transition between the benign and malignant areas.

DISCUSSION

Sampson has the credit of first pointing out the possibility of malignant transformation in ovarian endometriosis (Sampson 1924). He noted the similarity of some cases of primary ovarian carcinoma to endometrial carcinoma, and suggested that these may have arisen in ovarian endometriosis. In 1925, Sampson tried to prove his thesis by presenting 7 cases of primary ovarian carcinoma in which he

thought such an origin was possible. He also pointed out the difficulties of proving beyond doubt the origin of an ovarian carcinoma from pre-existent endometriosis: "Not only is there needed the actual demonstration of both cancer and endometrial tissue in the same ovary, the two bearing the same histologic relation to each other that cancer of the body of the uterus bears to the non-malignant portions of the endometrium of that uterus, in order to indicate the possible origin of ovarian carcinoma in this tissue but, to make it conclusive, it must be shown that the cancer arose in this tissue and that it is not invading it from some other source." Only one of Sampson's 7 cases can be said to have satisfied these criteria. However, this was enough to prove his thesis.

In spite of the writings of Sampson, some pathologists remained unconvinced with the possibility of malignant transformation in ovarian endometriosis. Among them, was the great pathologist Robert Meyer who also reviewed the slides of Sampson's cases (Thompson 1957). It was not till after about 20 years that other authentic cases began to appear and nowadays there is no doubt among all pathologists that ovarian endometriosis may turn malignant. To the criteria of Sampson, already mentioned, Scott (1953) suggested that "ideally, an additional qualification might be added: a microscopic section must show the benign endometriosis running into and continuous with the malignant epithelium," though he admitted that "for obvious reasons, this final limitation would be drawing much too fine a line." All these strict criteria have been fulfilled in several of the reported cases.

Malignant transformation has also been reported in other sites

of endometriosis, apart from the ovary: in the recto-vaginal septum (Ferreira and Clayton 1958, Lash and Rubenstone 1959, Dockerty et al. 1954), in the uterus (Greene 1961, Dockerty 1962), in the intestine (Weinrod et al. 1956), in the umbilicus (Novak and Woodruff 1962), in an obturator lymph node (Koss 1963). Several other examples can be sited.

To go back to our results, we have noticed in reviewing cases of ovarian endometriosis that anaplastic changes may be encountered, rarely, in benign cysts. We have also seen that endometriosis in postmenopausal women retains its capacity for active growth, given the proper stimulus, as happened in two of our cases in association with granulosa-theca cell tumours. Complying with the strict criteria for diagnosing the definite origin of a malignant tumour in ovarian endometriosis, we could diagnose four of our primary ovarian carcinomas to be arising in endometriosis.

The real incidence, however, may be higher than what can be gathered from these proven cases only. It is possible that besides these cases which retain a definite evidence of their origin, several others arising in the same way may have all traces of their origin obliterated by spread of the malignant growth. Malignant ovarian tumours histologically similar to endometrial growths are not infrequent. Search for such cases among our material showed that they accounted for about 12 per cent of all primary malignant tumours of the ovary. This group was found to include 12 cases of adenoacanthoma, 38 cases of adenocarcinoma of endometrioid pattern and two interesting cases histologically identical with the mesodermal mixed tumours of the uterus. Although an origin from endometriosis for these cases may be suggested, it is possible that they can arise

directly from the ovarian serosa without the intermediary of endometriosis.

Although Thompson (1957) concluded that almost all cases of primary ovarian adenoacanthoma arise in ovarian endometriosis, De Santo et al. (1959) interpreted their cases as a form of serous carcinoma. In our series, the finding of an adenoacanthoma in association with a mucinous cystadenoma in one case and with a serous cystadenocarcinoma in another case (Figures 45-48) may point to a possible common stem of origin for the three tumour types. An example of an adenoacanthoma in which evidence of endometriosis was lacking is shown in Figure 72.

The series of endometrioid carcinoma reported by Long and Taylor (1964) was the first series of this newly recognized group to be reported. The authors estimated the incidence of this group as about 17 per cent of all primary malignant tumours of the ovary and suggested an origin from the ovarian surface epithelium either directly or indirectly from benign endometriosis, implying that the ovarian serosa may retain the embryologic potential of the coelomic epithelium to differentiate into various tissues including uterine epithelium.

We would like to point out here some of the difficulties that may be encountered in classifying some ovarian carcinomas as of endometrioid pattern. Some of these difficulties have been pointed out by Long and Taylor (1964) and by Scully et al. (1966).

1. Occasional cases of ovarian carcinoma present difficulty in classification. These are usually those which are either undifferentiated or show a mixed pattern of differentiation. In undifferentiated tumours, a more thorough examination of other

parts may give a clue (Figures 16, 17). In tumours showing a mixed pattern (Figures 14, 15), the predominant pattern is usually accepted.

2. Endometrial carcinoma in the uterus, though commonly typical, is not always so. A mucinous type of endometrial carcinoma may rarely be noted (Haines and Taylor 1962). It is interesting to note that in malignant transformation in ovarian endometriosis, areas of mucinous carcinoma were noted by Moss and Runals (1948). Another unusual pattern in endometrial carcinoma of the uterus is the clear cell type suggestive of a mesonephric origin (Rutledge et al. 1965). It is interesting to note that in some cases of malignant transformation in ovarian endometriosis, this pattern has been noted (Plate, 1966, Gray and Barnes 1966, Scully et al. 1966). Scully et al. (1966) believe that the occurrence of clear cell carcinomas of the type often regarded as mesonephric in the endometrium and in ovarian endometriosis raises doubt concerning the mesonephric origin of tumours of similar appearance that are encountered in the ovary, broad ligament, cervix and vagina in the absence of a demonstrable relationship to endometriosis. In spite of these occasional aberrant differentiations in endometrial carcinoma of the uterus, in classifying cases of ovarian carcinoma as being of endometrioid pattern one has to consider only the typical and usual pattern.
3. The presence of endometrial carcinoma in the uterus in association with endometrioid carcinoma in the ovary raises doubt whether the ovarian tumour is primary or secondary. Several authors would accept that the ovarian tumour is primary if the endometrial carcinoma is disproportionately small, low-grade and no more than

minimally invasive of the myometrium (Long and Taylor 1964, Scully et al. 1966, Schueller and Kirol 1966). In 5 out of the 17 cases of primary ovarian endometrioid carcinoma, so classified by Scully et al. (1966), there was an associated endometrial adenocarcinoma in the uterus.

Apart from the possible relationship to endometriosis, recognizing the cases of endometrioid carcinoma of the ovary in a separate group may have a clinical value. Thus, a better prognosis has been described for these cases (Long and Taylor 1964, Schueller and Kirol 1966). Long and Taylor (1964) found a 5 year survival rate of 70 per cent in their series. A conservative management in cases of ovarian adenoacanthoma was compatible with long periods of survival (Thompson 1957). Again, there may be a place for the use of progestational drugs in the treatment of advanced cases of endometrioid carcinoma of the ovary, in the way they are used for advanced endometrial carcinoma of the uterus (Anderson 1965).

Ovarian tumours resembling the mesodermal mixed tumours of the uterus have been very rarely encountered. The recent trend in the histogenesis of these peculiar tumours is to regard them as of endometrial stromal derivation (Willis 1960). In the ovary, Willis (1960) expressed his view that they undoubtedly arise from foci of endometriosis. Cases of carcinosarcoma have in fact been occasionally reported in relation to ovarian endometriosis (Tuthill, 1938; MacFarlane and Pritchard, 1954; Marcella and Cromer, 1959). In one of our two cases, the origin from endometriosis was highly likely, although not definitely proved. Sternberg (1963), however, although admitting the theoretical possibility of the origin of these tumours from endometriosis, states that proof is lacking and classifies

them as of coelomic epithelial origin differentiating as a mixture of malignant Müllerian tissues.

In discussing the various types of malignant tumours to which the endometrium may give rise, the endometrial stromal sarcoma may also be mentioned. Sarcomatous transformation in endometriosis has been occasionally observed in the colon (Ferraro et al. 1956), in the rectum (Mallory, 1936) and in pelvic endometriosis (Pava et al. 1963). Cases have also been reported as stromal endometriosis, in uterine adenomyosis (Hunter and Lattig, 1958) and in ovarian endometriosis (Koller and Rygh, 1960; Benjamin and Campbell, 1960).

The origin of benign ovarian tumours in endometriosis has been suggested. Corner et al. (1950) and Scully et al. (1966) described mucinous cystadenomas arising in ovarian endometriosis.

In conclusion, it may be fair to say that in the present state of our knowledge the incidence of malignant transformation in ovarian endometriosis can only be put down in a vague and approximate way as lying somewhere between a minimum including only the definite cases and a maximum including all the cases showing a histological picture compatible with an endometrial origin.

Review of reported cases of malignant transformation in ovarian endometriosis

It is interesting to analyse the cases of definite malignant transformation in ovarian endometriosis in an attempt to define their clinical and pathological features. For this purpose, we added to our four definite cases, 48 other cases from the literature (Sampson 1925, case 5; Tuthill 1938; McCullough et al. 1946; Teilum 1946; Novak 1947; Kuzma 1947, case 1; Miller et al. 1947; Rauramo 1947;

Moss and Runals 1948; Corner et al. 1950, cases 4 and 5; Bacher and Hertzog 1951; Scott 1953, case 3; Fredrikson 1953, case 1; Hunter and Klein 1954, cases 1 and 2; Dockerty 1954, 2 cases; Postoloff and Rodenberg 1955; Kumar et al. 1955; Weinrod et al. 1956; Thompson 1957, cases 3, 5, 7, 10, 14 and 17; Greene and Enterline 1957, cases 1 and 2; Ferriera and Clayton, 1958; Wade 1960; Burt and Emson 1961, 3 cases; Concannon et al. 1962; Dockerty 1962, cases 5, 6, 7, 8 and 9; Freedman et al. 1964; Gray and Barnes 1965, cases 1, 2, 4 and 5; Ridley 1966; Plate 1966; Obstet. Gynec. Survey, 1966). This is not meant, however, to be a comprehensive review of all reported cases, though it probably includes all acceptable cases in the English literature. A summary of the features of these cases is given in Table X.

Age

The age of the patients ranged widely from 19 to 61 years.

19 years	1 case
20 - 29 "	3 cases
30 - 39 "	12 "
40 - 49 "	18 "
50 - 59 "	14 "
60 - 61 "	2 "
Age not stated	2 "



Endometriosis apparently may turn malignant long after the menopause and the cessation of ovarian activity. However, 16 out of the 52 cases were encountered in patients below the age of 40 years. This relatively high ratio of patients in the younger age groups is probably more apparent than real. This is because the slowly growing adenoacanthoma is more commonly encountered in the younger age groups and it is this type of tumour which is more liable to keep intact its histogenetic origin than the more rapidly growing

adenocarcinoma which is more common in the older age groups. The 19 year old patient had a carcino-sarcoma.

Parity

Twenty two patients out of 44 in whom the parity was known were nulliparous and none of the patients had more than three pregnancies.

Clinical features

The frequency of the leading symptoms was as follows:

Pain	23 cases
Lump or abdominal enlargement	19 cases
Uterine bleeding	12 cases
Urinary symptoms	6 cases
Asymptomatic	6 cases
Not stated	2 cases

Pain was the most frequent presenting symptom. Judging from the gross appearance of the endometrial cysts in these cases, where the malignant growth was almost invariably a more or less small mass, the pain apparently had a similar mechanism to the pain in cases of benign endometriosis, being caused by bleeding from the endometrium inside the cyst. Variations in the pattern of the pain can also be explained by variations in the pattern of bleeding from the malignant endometrium. Different from the periodic menstrual-related pain typical of benign endometriosis, the pain in these cases was frequently more or less continuous and when intermittent attacks were experienced, they were not related to menstruation. In some cases, the patient began to get attacks of pain after the menopause. Progressive increase in the severity of the pain was possibly also

related to an increase in the degree of bleeding. A practical suggestion out of these observations is that the possibility of malignant transformation should be considered in the patient known to have endometriosis, if the typical periodic pattern of pain changes, if the pain increases abruptly in severity or if attacks of pain start after the menopause.

In some cases, the cyst enlarged to form an abdominal lump or to cause a feeling of abdominal enlargement without causing any marked pain. Commonly, however, the patient complained of both pain and the feeling of a lump. The abdominal lump may be noticed to develop rapidly (Weinrod et al. 1956). The enlargement of the endometrial cyst was not caused by the bulk of the malignant growth which was almost invariably small in these cases but by distension due to bleeding from the malignant endometrium. The detection of an enlarging mass in a patient known to have endometriosis may direct attention to the possibility of malignant transformation (Dockerty 1962, case 7).

In one of our cases, and in two other cases (Teillum, 1946; Dockerty 1962, case 5), the patient presented with acute abdominal pain which proved on emergency laparotomy to be caused by rupture of an endometrial cyst that had undergone malignant transformation. Acute spontaneous rupture of ovarian endometrial cysts is a rare condition (Koskela, 1964). Pregnancy is the only significant factor predisposing to rupture which has been hitherto described (Scott, 1944). We would like to stress malignant transformation as another possible predisposing cause for acute rupture of endometrial cysts that should be carefully excluded in all such cases.

The three features of pain, an enlarging lump and spontaneous

rupture have the common underlying factor of intra-cystic bleeding from the malignant endometrium. A marked tendency for the malignant endometrium to bleed inside the endometrial cyst seems to play an important role in detecting these cases at an early stage. It is possible that some cases would lack such a feature, and in these as well as in malignant transformation developing in non-cystic endometriosis, the tumour may only be detected at a later stage.

An interesting symptom was uterine bleeding, often associated with the presence of endometrial polypi. Out of 30 cases in which the endometrial picture was reported, the endometrium was described as hyperplastic in 15 cases. This observation may possibly be looked upon as reflecting the response of the endometrium, in different locations, to a common stimulus, and we may recall here that in one of our cases of primary adenoacanthoma of the ovary, areas of squamous metaplasia were encountered in the benign uterine endometrium (Figures 73, 74). Whatever the explanation of this observation may be, uterine bleeding was apparently responsible for bringing a number of these cases to early notice.

In 6 cases the condition was asymptomatic. The case of Novak (1947) was operated upon because of the presence of a pelvic mass. The patient of Miller et al. (1947) had an asymptomatic mass. The case of Postoloff and Rodenberg (1955) was detected during examination for stress incontinence, and case 10 of Thompson (1957) during investigation of infertility. The appearance of a tender adnexal mass, in a patient known to have endometriosis, decided laparotomy in case 7 of Dockerty (1962). In one of our cases, operation was done for an asymptomatic pelvi-abdominal mass.

Again, it should be mentioned that these patterns of clinical

presentation were only those of the cases encountered at a sufficiently early stage to demonstrate their definite origin from endometriosis. It is possible that other cases without these symptoms may not be detected till a later stage when all evidence of their origin would have been eradicated.

Operative findings

At the time of operation, suspicion of the endometriotic origin of an ovarian malignant growth may be aroused by two factors which were almost constant in the reported cases: marked adhesions (not due to the extension of the growth) and the brownish colour of the fluid content of the cyst (observed when the cyst is opened after removal, aspirated or, as happened unfortunately frequently, inadvertently ruptured during removal). The presence of other stigmata of pelvic endometriosis will provide an additional feature but was not common in the reported cases.

Side of the tumour

In 23 cases the tumour was in the right ovary and in 17 cases in the left. In 7 cases, the tumour was bilateral. In only one of these (Corner et al. 1950, case 5) the bilaterality was interpreted as due to spread from one ovary to the other. The remaining cases were interpreted as separate primaries arising in bilateral endometriosis.

Gross pathological picture

The malignant endometrial cysts commonly showed one or more of the following gross features:

papillary areas	22 cases
solid areas	13 cases
abnormal large size	11 cases
spontaneous rupture	3 cases.

The presence of any of these features in an endometrial cyst should raise suspicion about malignant transformation. These features can also be correlated with the clinical picture. The tendency of the growth to project inside the cyst favours the development of intra-cystic bleeding with its previously discussed sequelae. The size of the cyst deserves further comment. A large size is very unusual for benign endometrial cysts; the largest encountered by Sampson was 15 cm. in diameter (Sampson 1925). Malignant endometrial cysts, on the other hand, may reach very large sizes, apparently due to recurrent bleeding from the endometrium. The cyst reported by McCullough et al. (1946) contained about 4,000 ml. of dark brown fluid, and in case 14 of Thompson (1957), the large cyst had to be emptied with a trocar before removal and 2,700 ml. of dark brown fluid were evacuated.

In 7 cases, however, the gross picture of the malignant endometrial cyst was not characteristic. This necessitates careful microscopical examination in all cases of ovarian endometriosis. It is also likely that in the advanced cases, endometriosis will not be apparent to the naked eye.

Microscopical picture

The types of tumour in the reviewed cases were as follows:

adenoacanthoma	25 cases
adenocarcinoma	25 cases
squamous cell carcinoma	1 case
carcinosarcoma	1 case

The frequency of the adenoacanthoma in these cases is probably more apparent than real. The adenoacanthoma is known to be commonly a slowly growing tumour (Thompson 1957). Hence, it is more likely to keep intact its relation to an endometriotic origin whereas a more rapidly growing adenocarcinoma is liable to overgrow the tissue of its origin and its relation to endometriosis may be missed. Again, the characteristic picture of the adenoacanthoma and its well-known relation to endometriosis are sufficient to stimulate the pathologist to make a careful examination of the case, where cases of adenocarcinoma are more liable to pass as cases of ovarian carcinoma, unless the relation to endometriosis is evident. In our material of primary malignant ovarian tumours, the ratio of the adenoacanthoma to the endometrioid adenocarcinoma was 1:3. If only the cases of definite origin in endometriosis are encountered, the ratio is 1:1. It is interesting to note also here that the adenoacanthoma was the more common type in the younger age groups. Thus, below the age of 40 years, 10 cases of adenoacanthoma were encountered, compared with five cases of adenocarcinoma.

Generally, the squamous component of the adenoacanthoma was histologically benign. However, McCullough et al. (1946) reported a squamous cell carcinoma arising in endometriosis and in the case of Moss and Runals (1948) areas of squamous cell carcinoma were encountered. In one of our cases, the squamous component was also malignant (Figure 67).

In the case of Rauramo (1947), a picture suggestive of Brenner tumour was described in some areas of the adenocarcinoma. In the case of Moss and Runals (1948), areas of mucous carcinoma were encountered. The adenocarcinoma in the case of Plate (1966) and

in case 2 of Gray and Barnes (1965) showed a clear cell pattern.

Prognosis

Data about the follow up of cases included in this review were rather fragmentary. Eleven patients were reported to be living and well for periods of five years or more. In three cases, the patient died after 6-18 months. In four other cases, a recurrence of the tumour was noted. In two cases, this involved the region of the broad ligament and occurred in the second year (Bacher and Hertzog 1951, and one of our cases). In two other cases, the recurrence involved the intestine and occurred after five years (Weinrod et al. 1956; Dockerty, 1962, case 8).

In speculating on the possible prognosis in these cases, several points may be considered:

1. The tumour may well be detected at an early stage becoming symptomatic for reasons we have already set out.
2. A low grade of malignancy is common in the cases that are recognized although, as previously pointed out, there is the likelihood that the higher grades of malignancy are missed due to the overgrowth of any traces of their origin.
3. Adhesions due to endometriosis may be extensive and may make the operation formidable and possibly incomplete. Inadvertent rupture of the cyst during removal was not infrequent in the reported cases.
4. There is suggestive evidence in these cases that malignancy may develop in other sites of endometrial tissue, either simultaneously or at a later date. In 5 of the reported cases, bilateral tumours were considered to have developed independently in endometriosis involving both ovaries. The development of a separate

adenoacanthoma in the uterus was reported by Freedman et al. (1964). Dockerty (1954) suggested that independent foci of neoplasm in the uterus and ovary may account for some of the cases where adenoacanthoma is found in both the uterus and ovaries. In the cases of Weinrod et al. (1956) a separate adenoacanthoma developed in intestinal endometriosis five years after the removal of an ovarian adenoacanthoma. In our case 1, the tumour removed one and a half years after the original operation was interpreted as a malignant transformation in an endometriotic lesion left from the previous operation. Thompson (1957) suggested that the presence of an adenoacanthoma in multiple sites may depend more on the ability of the normally placed endometrium and the ectopic endometrial implants to respond to factors which incite adenoacanthomatous change, than on the ability of the adenoacanthoma to metastasize.

These points raise the question of the extent of operation in these cases. The detection of these growths at an early stage, their usually low grade of malignancy as well as the young age of many of the patients may tempt towards a more conservative approach (Thompson 1957). The risk of leaving endometrial tissue, normal or ectopic, in which an endometrial carcinoma may subsequently develop or may be already present at a very early stage is possibly small but should probably be also taken into consideration.

SUMMARY

In an approach to the problem of malignant transformation in ovarian endometriosis, we have reviewed a series of 592 cases of ovarian endometriosis and a series of 418 primary malignant ovarian tumours, encountered during a period of 10 years. Anaplastic changes were encountered in four benign endometrial cysts. Malignant tumours histologically similar to endometrial growths were encountered in 52 cases. Of these, only four could be shown to have a definite endometriotic origin. Analysis of these and of 48 cases from the literature suggests that some clinical and pathological characteristics were common in these cases. The relation of these characteristics to the detection of the growths at an early stage is discussed.

TABLE X

SUMMARY OF CLINICAL AND PATHOLOGICAL FEATURES IN REPORTED CASES OF MALIGNANT TRANSFORMATION IN OVARIAN ENDOMETRIOSIS

author	age	parity	leading symptoms	side	gross characteristics	microscopical picture	uterine endometrium	follow up
Sampson (1925) case 5	46	0	- pain -abdominal distension	-	papillary	adenocarcinoma	polypoid cystic	-
Tuthill (1938)	19	0	- pain - abdominal lump	right	- large - firm areas	carcinosarcome	-	recurrence & death after 6 months
McCullough et al.	61	0	- abdominal distension	right	- large	squamous cell carcinoma	-	recurrence & death after 6 months
Teilum (1946)	42	1	acute pain	left	ruptured	adenoca cinoma	polypoid hyperplastic	-
Novak (1947)	32	0	asymptomatic pelvic mass	left	none	adenoacanthoma	-	living and well for 12 years
Kuzma (1947) case 1	42	3	pain	right	solid nodule	adenoacanthoma	hyperplastic	-
Miller et al. (1947)	42	2	asymptomatic mass	right	large	adenocarcinoma	- polyp - hyperplastic	-
Rauramo (1947)	52	2	- pain - abdominal lump	-	- large - solid nodules	adenocarcinoma ? malignant Brenner	normal	-

author	age	parity	leading symptoms	side	gross characteristics	microscopical picture	uterine endometrium	follow up
Moss and Runals (1948)	45	3	abdominal fullness	right	- large - papillary	-adenocanthoma -squamous cell carcinoma - mucous cell carcinoma	- polyp - cystic	-
Corner et al. (1950) case 4	54	0	abdominal lump	right	papillary	adenocarcinoma	- polyp - cystic hyperplasia	recurrence and death after 18 months
case 5	50	1	- urinary	bila-teral	papillary	adenocanthoma	atrophic	living and well for 6 years
Bacher & Hertzog (1951)	48	-	pain	bila-teral	none	adenocarcinoma	atrophic	recurrence after 16 months
Scott (1953) case 3	50	0	uterine bleeding	right	solid areas	adenocarcinoma	polyp	-
Fredrikson (1953) case 1	54	1	-urinary symptoms -abdominal distension	bila-teral	papillary	adenocarcinoma	-	-
Hunter & Klein (1954) case 1	44	3	abdominal lump	right	papillary	adenocarcinoma	-	-
case 2	54	0	uterine bleeding	left	papillary	adenocarcinoma	polyps	-

author	age	parity	leading symptoms	side	gross characteristics	microscopical picture	uterine endometrium	follow up
Dockerty (1954)	-	-	-	-	-	adenocanthoma	-	-
Postoloff & Rodenberg (1955)	50	1	none	right	nodular projections	adenocanthoma	- cystic polypoidal	-
Kumar et al. (1955)	39	1	- abdominal lump - pain	left	papillary	adenocarcinoma	hyperplasia	-
Weinrod et al. 1956	55	1	- vaginal discharge - abdominal lump	right	large	adenocanthoma	polyp	adenocanthoma of intestine after 5 yrs
Thompson (1957) case 3	37	0	uterine bleeding	right	firm mass	adenocanthoma	normal	living and well for 8 years
case 5	27	0	pain	bilateral	none	adenocanthoma	normal	living and well for 6 years
case 7	37	0	uterine bleeding	left	none	adenocanthoma	-	living and well for 5 years
case 10	33	0	none	right	solid	adenocanthoma	normal	living and well for 4 years

author	age	parity	leading symptoms	side	gross characteristics	microscopical picture	uterine endometrium	follow up
case 14	54	2	abdominal enlargement and discomfort	right	- large papillary	adenocarcinoma	atrophic	living and well for 7 years
case 17	28	1	pain	right	none	adenocarcinoma	-	-
Greene & Enterline (1957) case 1	51	3	uterine bleeding	right	papillary	adenocarcinoma	hyperplasia	-
case 2	52	0	abdominal lump	right	papillary	adenocarcinoma	hyperplasia	-
Ferriera & Clayton (1958)	40	2	pain	left	large	adenocarcinoma	normal	-
Wade (1960)	37	0	heaviness in abdomen	right	papillary	adenocarcinoma	normal	living and well for 21 months
Burt & Emson (1961) case 1	49	1	uterine bleeding	bilateral	none	adenocarcinoma	-	living and well for 10 years
case 2	49	0	- pain - abdominal lump	left	- large - solid area	adenocarcinoma	-	living and well for 6 years
case 3	33	1	pain	right	irregularly thickened wall	adenocarcinoma	-	living and well for 16 months

author	age	parity	leading symptoms	side	gross characteristics	microscopical picture	uterine endometrium	follow up
Concannon et al. (1962)	38	2	- pain - uterine bleeding	bilateral	papillary	adenocanthoma	normal	living and well for 25 months
Dockerty (1962) case 5	34	parous	acute bleeding	right	- ruptured - solid nodule	adenocarcinoma	-	-
case 6	36	0	- abdominal bloating - urinary symptoms	left	- large - semisolid mass	adenocarcinoma	-	living and well for 14 years
case 7	40	0	none	right	papillary	adenocarcinoma	-	living and well for 5 years
case 8	43	0	abdominal discomfort	right	none	adenocarcinoma	-	recurrence after 6 yrs
case 9	40	-	pain	right	solid nodule	adenocanthoma	-	-
Freedman et al. (1964)	51	0	- urinary symptoms - abdominal discomfort	left	papillary	adenocanthoma	adenocanthoma	-
Gray & Barnes (1965) case 1	29	0	- uterine bleeding - pain	bilateral	projecting teral growth	adenocanthoma	atypical hyperplasia	well for 4 years
case 2	60	-	enlarging lump	left	- large - solid part	clear cell adenocarcinoma	normal	well after 6 years

author	age	parity	leading symptoms	side	gross characteristics	microscopical picture	uterine endometrium	follow up
case 4	42	-	pain	left	- large - papillary	adenocarcinoma of secretory type	hyperplasia	-
case 5	46	-	uterine bleeding	left	- papillary	adenocarcinoma	-	-
Ridley (1966) case 2	38	1	- uterine bleeding - urinary symptoms	left	papillary	adenocarcinoma	normal	living and well for one year
Plate (1966)	41	2	pain	left	papillary	clear cell carcinoma	-	-
Obstet & Gynec. Survey (1966)	56	2	- pain - menorrhagia	-	indurated area	adenocarcinoma	-	-
Present series case 1	52	0	- pain - abdominal lump - urinary symptoms	left	papillary	-adenocarcinoma atrophic - squamous cell carcinoma	-	recurrence after 1 yr.
case 2	47	-	asymptomatic pelvi-abdominal mass	right	friable papillae	adenocarcinoma	normal	living and well for 3 years
case 3	46	0	- acute pain - abdominal lump	left	papillary	adenocarcinoma	normal	-
case 4	37	0	pain	left	papillary	adenocarcinoma	normal	-

CHAPTER 2THE OCCURRENCE OF GRANULOSA AND THECA CELL TUMOURS IN
CLINICALLY NORMAL OVARIES

The frequency with which granulosa and theca cell tumours may be present for long periods without causing clinical enlargement of the ovary is not generally realized. During the review of the ovarian tumours in our series we encountered several cases of this type. For their special interest, from both the clinical and pathological point of view, we went through the files beyond the period of 10 years included in the review. A series of 25 such tumours will be the subject of the present discussion. The clinical records of the patients were traced, their clinical features analysed, the histological sections were reviewed and other sections were cut for further study in several of the cases.

Incidence

Of 91 cases of granulosa and theca cell tumours examined in the pathology laboratory of the Department of Obstetrics and Gynaecology of the University of Edinburgh since 1950, 25 tumours were encountered in ovaries that were normal-sized or only slightly enlarged, and were judged clinically to be normal.

Summary of the clinical features

The age of the patients in the 25 cases of small tumours ranged from 47 to 80 years with only one patient below the age of 50 years. In the total series of 91 granulosa and theca cell tumours of all sizes, age ranged between 17 and 86 years with 23 patients below the age of 50 years.

Eleven patients were nulliparous and all were postmenopausal

for periods varying from 2 to 31 years.

With the exception of 3 cases, the presenting symptom was postmenopausal bleeding. Of these 3 patients, one was operated upon for the presence of large uterine fibroids, another had a large twisted fibroma of the opposite ovary and the third had an adenocarcinoma of the opposite ovary. The duration of the history of postmenopausal bleeding varied from two weeks up to 6 years. Delays were largely due to reluctance on the part of the gynaecologist to operate in the absence of a palpable tumour. Thus, only eleven of the patients had a laparotomy within one year of the start of the bleeding, 6 patients were operated upon in the second year, 3 in the third year, one after 4 years and one after 6 years.

The management of the patients presenting with postmenopausal bleeding followed several lines. In 2 patients, laparotomy was the first line of treatment. In 12 patients, laparotomy was performed after an initial curettage showing endometrial hyperplasia (7 cases) or carcinoma (5 cases). In two patients, curettage was performed and hysterectomy was done when the bleeding recurred. In 4 patients, the decision for laparotomy was only taken after 2 or 3 curettings had been performed. In 2 patients, after an initial curettage, deep X-ray therapy to stop ovarian function was given. In both cases, the treatment failed. One of these two patients had the X-ray treatment repeated 3 times and was only operated upon after 3 years when an early adenocarcinoma of the endometrium was also discovered. The second of these two patients had a laparotomy after intermittent bleeding for 6 years.

It is interesting to note that a history of radiation menopause

was present in 3 other cases in this series. One patient had a radium-induced menopause for the treatment of dysfunctional premenopausal bleeding 18 years before. Another patient had the same treatment 31 years before the occurrence of postmenopausal bleeding and the detection of an endometrial adenocarcinoma. The third patient received radiotherapy for the treatment of fibrosarcoma of the groin at the age of 28 years. Her periods stopped since then and she presented 24 years later with uterine bleeding and endometrial hyperplasia.

Oestrogen assays were available in 2 of the patients with postmenopausal endometrial hyperplasia. In one patient, aged 75 years and menopausal for 27 years, the 24 hours urine volume contained a total of 17.8 microgram oestrogens. In the second patient, 66 years old and 10 years postmenopausal, the total oestrogen values were 38.7 microgram per 24 hours urine volume. The total mean urinary oestrogen excretion in the normal postmenopausal patient is of the order of 5.8 microgram per 24 hours with a range of 3.1 to 8.1 microgram (Kellar et al. 1959).

Summary of pathological features (Figures 75-92)

All the tumours were unilateral, with the exception of one case of bilateral thecoma. Seventeen of the tumours were thecomas and 8 were granulosa-theca cell tumours. In our total series of 91 cases, only 28 were thecomas. The appearance described as luteinization was more frequently observed and also more marked than in the large tumours (Figures 80, 87, 89). In 3 of the cases, the tumour was almost completely luteinized. In spite of their small size, the tumours often showed evidence of old fibrosis, and hyalinization was usually marked and in some cases replaced a large

part of the tumour. The ovary, apart from the tumour, was commonly the seat of a variable degree of cortical stromal hyperplasia. In one case, foci of lutein cells were included in the hyperplastic ovarian stroma (Figure 92). Hyperplasia of the hilus cells was prominent in one case. A small fibroma was also present in the same ovary in one case. Corpora-albicantia-like structures were prominent in some of the cases of granulosa cell tumours (Figure 78).

In 3 patients, the endometrium was inactive. These were the three patients, already mentioned, who presented with other large tumours in the uterus or opposite ovary. In 15 cases, the endometrium showed marked proliferative activity or cystic hyperplasia (Figures 77, 81, 90, 91). In the remaining 7 cases, endometrial carcinoma was present. In 5 out of these 7 cases, the ovarian tumour was a thecoma. It is also worthy noting that in the total series of 91 cases of granulosa and theca cell tumours, eleven cases of concomitant endometrial carcinoma were encountered. In other words, in 7 out of eleven cases in which a granulosa or theca cell tumour was associated with endometrial carcinoma, the ovary showed no clinical enlargement. Two of the cases of endometrial carcinoma in this series are of special interest. Endometrial curettage, two years before in one patient and 3 years before in the other patient, showed postmenopausal active hyperplasia. The second patient received in the interval deep X-ray therapy to stop ovarian function.

DISCUSSION

Although granulosa and theca cell tumours of very small size have been occasionally noted in previously published series (Biggart and Macafee 1955), they apparently received no special attention. Our study of a group of 25 such cases revealed some interesting features worthy of discussion.

In our material, 25 out of 91 granulosa and theca cell tumours were detected in ovaries showing no clinical enlargement. The real incidence of these small tumours may even be higher since they are more liable to be missed than the larger tumours, especially if they are hormonally inactive. The relative frequency of these tumours should direct attention to their possible presence in the postmenopausal patient presenting with evidence of abnormal oestrogenic activity, even when the ovaries are palpably normal.

Several observations suggest that these cases do not simply represent early tumours but rather a variant of granulosa and theca cell tumours characterized by a very slow and possibly limited capacity for growth. Thus, the tumours were encountered not at an earlier age but rather at a later age than the larger tumours. Furthermore, the history of these patients often revealed evidence of long standing abnormal oestrogenic activity before operation. Pathological examination also often showed a pronounced degree of fibrosis and hyalinization in the tumours. In the present state of knowledge, factors governing the rate and capacity of growth in tumours are not well understood. However, two pathological features in our series are worthy of note in this connection. The first is the predominance of the thecal type of tumour and this is in accord

with the observation that thecomas are generally more slowly growing and relatively more benign than the granulosa type of tumour. The second feature is the more frequent and more marked appearance of luteinization in these small tumours. Since this distinctive histological appearance is probably indicative of steroid storage in the cells, it may be suggested that there is a correlation between a low potential for growth and a capacity of the cells for steroid storage. It is interesting in this aspect to note that the so-called lipoid cell tumours of the ovary are usually of a small size (Haines and Taylor, 1962).

The association of endometrial carcinoma with feminizing mesenchymal tumours has been reported, the figures varying from 3 to 27 per cent (Hertig and Gore, 1963). In our material, the incidence of endometrial carcinoma was disproportionately more high with the small tumours than with the larger tumours. Thus, 7 cases of endometrial carcinoma were encountered among the 25 cases of small tumours, whereas in the other 66 cases of granulosa and theca cell tumours in our material, only 4 cases of endometrial carcinoma were encountered. Several factors may be considered to explain this relatively high frequency. One of these may be the predominance of thecomas in these small tumours, since thecomas are more notable than the granulosa cell tumours for this association (Greene, 1957). The older age of the patients may be another factor. It is also possible that more of these small tumours are detected when the endometrium is malignant than when it is benign because operation is less readily undertaken when the endometrial curettings are benign. However, despite all these possible factors, the frequency of the association is probably not fully explained. It may also be

mentioned that Greene (1957) noted that 7 out of 13 mesenchymomas in association with endometrial carcinoma were not detected before operation. Here, a possible explanation may be ventured; these small tumours escaping clinical recognition for a long time are expected to exert a prolonged oestrogenic effect on the endometrium, more than is possible with the larger rapidly growing types. In two cases in this series, the progression from postmenopausal hyperplasia of the endometrium to adenocarcinoma was noted after 2 years in one patient and 3 years in the other. For all probability, the small tumour was present over these periods. The possibility of the presence of a small undetected functioning tumour of the ovary in a case of endometrial carcinoma is worthy of remembering. Such a condition may be responsible for failure of treatment by radium as it probably was in a case mentioned by Sparling (1950).

The possibility that radiation may play a role in the development of feminizing ovarian tumours in the human as well as in the experimental animal has been suggested (McKay et al. 1953). It was therefore of interest to note that 5 patients in this series received radiotherapy, usually to stop ovarian function, before the discovery of the tumour. It is possible, however, that some of the patients may have had the tumour at the time of the radiotherapy. Indeed, this appears to be the most plausible explanation in 2 of our cases as well as in some other similar reported cases (Maxwell, 1956; Sparling, 1950; Pratt, 1950). In other words, the presence of the undetected functioning tumour was the cause of the radiotherapy and not the reverse. It is important to remember that a patient bleeding around the time of the menopause and showing evidence of oestrogenic activity may have an undetected functioning

ovarian tumour, especially if a standard dose of radiotherapy fails to stop ovarian activity. In 2 other cases in this series, the long interval of amenorrhoea between the induction of menopause for abnormal uterine bleeding and the presentation of the tumour makes it more probable that the tumour has developed after the menopause. In these 2 cases, however, it may be argued that the etiological relation, if any, is with the ovarian dysfunction at the time of the menopause and not with the irradiation.

SUMMARY

The frequency with which granulosa and more particularly theca cell tumours may be present for long periods without causing clinical enlargement of the ovary is not generally realized. In our material, 25 out of 91 tumours were of this type. These small tumours appear to have a very slow and possibly limited capacity for growth but are endocrinologically active. Endometrial carcinoma was present in 7 out of our 25 cases. A history of ovarian irradiation was present in 5 cases. The significance of these observations is discussed.

CHAPTER 3OBSERVATIONS ON VERY RARE TUMOURS OF THE OVARY

In the first chapter of this thesis, we tabulated ovarian tumours according to frequency into common, uncommon, rare and very rare tumours. As a rough index, it was pointed out that generally more than one thousand consecutive cases of ovarian tumours are needed to get one of the tumours in the very rare group.

In this chapter, a detailed account of the very rare ovarian tumours which we diagnosed during our review will be given. The rarity of the tumours, the lack of clarity about their status and the diagnostic difficulties to which they may give rise justify their grouping together here for discussion.

The tumour types that will be dealt with are the following:

1. Primary mesodermal mixed tumours of the ovary.
2. Malignant Brenner tumour.
3. Malignant teratoma.
4. Malignant adenofibroma.
5. Mesoblastoma (Teillum).

PRIMARY MESODERMAL MIXED TUMOURS OF THE OVARY

It is very rare to encounter in the ovary tumours resembling the mesodermal mixed tumours of the uterus. A search of the literature is hampered by the multitude of names under which these tumours may have been reported. The case of Katsunuma et al. (1959) was stated to be the first case reported under the title of mixed mesodermal tumour of the ovary. Other authors have mentioned cases of this type but without giving further details (Willis, 1960; Sternberg, 1963). It is also possible that the cases of ovarian carcino-sarcoma reported by Tuthill (1938), MacFarlane and Pritchard (1954) and Marcella and Cromer (1959) belong to the same group. Cases reported as rhabdomyosarcoma of the ovary (Sandison, 1955; Payan, 1965) possibly also belong to the same group.

The diagnosis of mesodermal mixed tumours in the ovary is based primarily on the same criteria for the similar uterine tumours (Taylor, 1958). However, two more criteria should be added in the case of the ovary: firstly, the possibility of a spread from a uterine primary should be excluded and secondly, care should be taken not to mistake cases of teratoma with these tumours. Mesodermal mixed tumours are menodermal while teratomas are tridermal. Other teratomatous elements should be looked for. This difficulty does not arise in the uterus where teratomas are practically unknown.

As regards the pathogenesis of these rare tumours in the ovary, Willis (1960) expressed his view that they undoubtedly arise from foci of endometriosis. In one of our own two cases, an origin from endometriosis was highly likely though not definitely proved. Sternberg (1963), on the other hand, although admitting the

theoretical possibility of the origin of these tumours from endometriosis, states that proof is lacking and classifies them as of coelomic epithelial origin differentiating as a mixture of malignant Müllerian tissues.

The following two cases of mesodermal mixed tumours of the ovary were encountered in our series of 1734 ovarian tumours. The two cases were originally diagnosed as ovarian carcinoma but a more thorough examination during our review allowed us to reclassify them as primary mesodermal mixed tumours of the ovary.

Case 1: This patient, aged 59 years, para 0 and 19 years postmenopausal presented with an abdominal mass. At operation, the presence of ascites, together with a twisted tumour of the left ovary, more than 20 cm. in diameter, was noted. A total hysterectomy with bilateral salpingo-oophorectomy was carried out. An extensive right pleural effusion, present before the operation, cleared gradually after removal of the tumour. On pathological examination, the uterus was found to be normal and the endometrium showed a mild degree of proliferative activity. The right ovary and the tubes were unremarkable. The sections available from the tumour showed three different pictures. One part had the appearance of a benign adenomyoma (Figure 93). Another part showed the transformation of the stroma of the tumour to a loose myxomatous type, with clusters and individually dispersed malignant cells and also with areas of degenerate chondrous tissue (Figure 94). In a third part, the epithelial elements showed the pattern of endometrioid carcinoma (Figure 95). Extensive areas of haemorrhage and necrosis were seen in the tumour, possibly due to the torsion.

Although in this case the origin of the mesodermal mixed tumour

from the associated endometriosis was highly probable, we could not, in the absence of the original specimen, demonstrate to our satisfaction a transitional zone between the two lesions.

Case 2: This patient, aged 64 years, para 0 and 20 years postmenopausal, was diagnosed to have a mobile ovarian tumour. Total hysterectomy with bilateral salpingo-oophorectomy was performed. The tumour was confined to the left ovary with no evidence of spread. However, the patient died 4 months after the operation with ascites and liver enlargement. On pathological examination, the endometrium was atrophic with several cystic inactive glands. The cervix showed superficial infection. Both tubes and the right ovary were histologically normal. The left ovarian tumour showed several cystic areas containing blood stained fluid but was largely solid. Microscopical examination showed the presence of malignant epithelial elements in a richly cellular stroma. The epithelial elements showed largely a well differentiated glandular pattern (Figure 96). The stroma showed the picture of broad irregular bands, interlacing in various directions. In the interstices collections of large cells, rounded, oval or racket shaped and with abundant eosinophilic cytoplasm were encountered here and there (Figure. 97). These cells were interpreted as rhabdomyoblasts. Further sections stained with Heidenhain's iron haematoxylin demonstrated cross striated muscle fibres in several areas (Figure 98).

MALIGNANT BRENNER TUMOUR

Brenner tumours were considered as entirely benign until 20 years ago, when Von Numers (1945) reported the first two possible cases of malignant forms of the Brenner tumour. Several cases were subsequently reported. Idelson (1963) reviewed the literature, analysed the 25 cases reported and reported one case of his own. At least two other cases were reported after Idelson's review (Arffman, 1962; Shay and Janovski, 1963). It is doubtful, however, if all the reported cases were really authentic. Epithelial clumps in an ovarian carcinoma may simulate the appearance of the Brenner tumour. Figure 99 shows such an example. Strict criteria should therefore be fulfilled before this diagnosis is made (Idelson, 1963), most important among which is that definitely benign parts of the tumour should be demonstrated.

Variable microscopical pictures have been described in the previously reported cases. With the exception of two cases (Behrens, 1953; Bamforth et al. 1951) the malignancy arose from the epithelial elements. In the case of Behrens (1953) benign glands were present but atypical mitoses and pleomorphism were displayed by the stromal element. In case 1 of Bamforth et al. (1951) malignancy was shown by both epithelial and stromal elements. Different patterns were described for the epithelial malignancy in the reported cases. Some cases showed a squamoid pattern with the malignant epithelial cells aggregated in small and large sheets. In other cases, adenomatous propensities were marked in the malignant epithelial areas. In still another common pattern, large cystic structures were noticeable. These were lined by atypical Brenner epithelium and sometimes showed papillary projections.

Often the tumours displayed pictures far from uniform and displayed a mixture of different patterns.

The endometrium was noted to be hyperplastic in some of the cases and abnormal uterine bleeding was also encountered. In the case of Shay et al. (1963) a malignant Brenner tumour was reported in association with endometrial carcinoma.

The following case is the only case I encountered in our material. The original diagnosis was an adenocarcinoma. This case showed the presence of the benign and malignant Brenner tumour in one section with evident transitions.

Case report: Mrs. A.F., aged 80 years was admitted to hospital with the complaint of lower abdominal pain of 2-3 months duration. Her periods had ceased at the age of 40 years but during the past 12 years she had more or less regular episodes of bleeding which she interpreted as a return of the periods. She was a para 3. She had no alimentary symptoms. She had some difficulty in passing urine but no dysuria. Micturition was slightly more frequent. Her appetite was good and her weight was steady. Examination showed the presence of a pelvi-abdominal tumour. Laparotomy was performed and a tumour of the left ovary was removed together with a supravaginal hysterectomy and right salpingo-oophorectomy.

The tumour was about 9 inches in diameter, with its surface lobulated, smooth and glistening. Mild adhesions were present. The tumour was cystic but with solid areas near the base (Figure 100). The fluid content was yellow and rather gelatinous. The uterus and opposite ovary and the tubes were normal.

Microscopical examination of the solid areas showed the typical appearance of benign Brenner tumour. The cell islands looked mostly inactive with peripheral hyaline degeneration and/or central liquifaction (Figure 101). The cystic part of the tumour, on the other hand, showed the picture of a papillary cystadenocarcinoma, with the multi-layered malignant epithelium showing a marked structural similarity to the Brenner pattern (Figure 102). Evidence of stromal invasion was present.

MALIGNANT TERATOMA

In the first chapter of this thesis, the classification of cases of ovarian teratoma was outlined. A distinction between malignant transformation in a benign cystic teratoma and the malignant teratoma (the solid malignant teratoma) was made. Here we are going to describe an unusual case of solid malignant teratoma in which the component tissue elements were of mature adult type. The malignant potential of the tumour was proved by recurrence of the growth.

Tumours of similar nature have been reported by other authors. Peterson (1956) summarized 10 reported cases. Although in 3 of these, implants of neuroglial tissue were present in the peritoneal cavity, Peterson regarded these cases as benign and labelled them as solid histologically benign teratomas of the ovary. Benirschke et al. (1960) reported 3 cases of similar nature but in whom the tumours spread widely and caused the death of two patients. It is therefore possible that the long survival of the patients reviewed by Peterson was largely due to the complete removal of the tumour and not to its benign potential. On the evidence of the cases of Benirschke et al. (1960) and of our own case, we prefer to label these cases as solid well-differentiated malignant teratomas, rather than benign teratomas.

Case report: The patient was a 14 years old girl who has never menstruated and who has always been small for her age. She was quite well until several weeks before admission to hospital, when she first noticed a dull pain in the right side of her abdomen. Her abdomen was also noticeably gradually enlarging. Her appetite was not so good lately. Nausea and some vomiting were present for

the last few days. Her bowels were regular. She had occasional nocturnal urinary frequency. She had a tonsillectomy at the age of 10 years. Her 5 brothers and 2 sisters were all well.

The patient, on examination, had no axillary or pubic hair and her breasts were not well-developed. Heart and chest were free. The abdomen was considerably distended to a girth of 24 inches by a large tensely cystic tumour arising from the pelvis and extending to within a finger breadth of the xiphi-sternum. Both lung fields were clear on X-ray examination. Intravenous pyelography showed that both ureters were compressed by a large tumour with patchy calcification.

Lapatoromy was carried out. Free fluid was present in the peritoneal cavity. A right ovarian semi-solid tumour, 8 x 6 x 6 inches with a large pedicle was present. The left ovary was normal. The tumour and the right tube were removed.

Pathological examination of the tumour showed the appearance of a well-differentiated teratoma containing a wide range of tissue forms: brain, ependyma, choroid plexus (Figure 103), squamous ciliated and mucinous epithelium, hair follicles, fat, nerve fibres and smooth muscle. All these tissues were of adult mature type.

Six months later, the patient presented with a firm mass on the left side of the abdomen, reaching almost to the level of the umbilicus. The patient was still amenorrhoeic and the abdominal mass was apparently asymptomatic. The patient was admitted again to hospital and laparotomy was performed. The uterus was found to be pushed up out of the pelvis by a large multilocular cyst with solid areas and about 4 inches in diameter, lying in the pouch of Douglas. The left ovary, tube and uterus appeared to be

normal. Fine granular deposits were present on the peritoneum between the uterus and bladder and on the greater omentum. The liver was normal. The tumour in the pouch of Douglas was removed and biopsies were taken from the greater omentum and the pelvic wall peritoneum.

Pathological examination of the tumour again showed only well-differentiated recognizable adult tissues, including bone, cartilage, squamous and mucinous epithelium. The peritoneal biopsies were composed only of neuroglial tissue (Figure 104).

MALIGNANT ADENOFIBROMA

The potential of malignancy in the adenofibroma and cystadenofibroma of the ovary has been already discussed in the first part of this thesis. A distinction was drawn between malignant transformation in the cystic and in the solid part of the tumour. Carcinomatous transformation in the glandular elements in the solid part of the tumour is a very rare occurrence. The following report discusses such a case.

Case report: The patient was 72 years old, para 0 and menopausal for 22 years. She was first seen with an attack of deep-seated thrombophlebitis and pulmonary embolism. On abdominal examination, a big mobile cystic mass, reaching up to two fingers above the umbilicus was discovered. She had no vaginal bleeding. At operation, a left ovarian tumour together with the uterus and right appendages were removed. There was no ascites and no evidence of peritoneal or liver metastases.

On pathological examination, the uterus was found to be atrophic with few seedling fibroids. The endometrium showed a moderate degree of proliferative activity. The cervix was benign. The tubes and right ovary were normal. The left ovarian tumour showed the appearance of unilocular cyst, 14 x 9 x 9 cm. with a solid rounded firm mass, 7.5 x 5 cm. arising from part of the cyst wall and projecting in the cavity. Microscopically, the cystic part showed a lining of benign cubical epithelium with occasional stunted papillae and psammoma bodies (Figure 105). The solid part of the tumour showed 4 different pictures:

1. benign small glands widely dispersed in fibrous tissue, typical of benign adenofibroma (Figure 106).
2. small glands crowded together in large compact clusters (Figures 107, 108).
3. loss of gland pattern and arrangement of the cells in clumps and columns, surrounded by a fibromatous stroma. The cells show malignant characteristics and the appearance may suggest superficially a malignant Brenner tumour (Figure 109).
4. Anaplastic looking cells dispersed singly or in small clusters in the fibromatous stroma (Figure 110).

The four histological pictures could be seen in the same section and there was no doubt about the transition from one part to another. The stroma of the tumour was largely fibromatous but occasional areas of peri-epithelial thecal reaction were marked (Figure 110).

Figures 111 and 112 show a similar picture in another tumour which we encountered but in which detailed examination was not possible.

MESOBLASTOMA OF TEILUM

Since Schiller's description in 1939 of the tumour which he designated as mesonephroma, the status of this tumour has always been the source of confusion. The opinion today is still divided. Some authors would not recognize the tumour as an entity (Willis, 1960; Haines and Taylor, 1962). Others maintain the mesonephric origin as suggested by Schiller (1939) or a meso-metanephric origin (Novak and Woodruff, 1963). Others claim a germ cell origin (Teilum, 1965). It seems that this divergence of opinion is partly due to different authors speaking about different tumour types and partly due to much stress being laid on certain structures in the tumour which are not always present and which suggested different appearances to different authors. These are the glomeruloid structures of Schiller and the endodermal sinuses of Teilum. An epithelial tumour of the ovary may show the picture described as mesonephroma (Figures 113, 114). The picture can be striking in some cases but it is doubtful whether or not a mesonephric origin underlies its development. The tumour discussed in the writings of Teilum is of a different nature. Its main characteristic is the primitive mesenchymal type of stroma and the paucity of epithelial elements. At least a superficial resemblance to embryonic mesenchyme can be seen. Teilum's interpretation of the tumour as an extra-embryonic mesoblastoma may well be correct, though the interpretation of the epithelial spaces in the tumour as homologous with the endodermal sinuses or as yolk sac endoderm (Teilum, 1965) is debatable.

We encountered two tumours in our series which answer to the description of Teilum's tumour. In both cases, the patient was below the age of 20 years, a common characteristic for this type of tumour, in contrast to the malignant epithelial tumours of the ovary.

Case 1: This girl, aged 17 years, was first seen complaining of abdominal enlargement and a sense of tightness of 16 months duration. Her periods, starting at the age of 13 years, were somewhat irregular. She was found, on examination, to have large bilateral pleural effusions, ascites and an abdominal mass the size of 20 weeks pregnancy. Laparotomy was performed and a large right sided ovarian tumour was removed. The tumour was already ruptured before the abdomen was opened. The patient was allowed home after 10 days.

The tumour was firm and solid and measured 19 x 12 x 11 cm. Its surface was slightly lobulated. On section, it was partly solid and partly cystic, multiple small cysts being present. The solid areas had a rather gelatinous appearance and were yellowish apart from extensive blood staining. On microscopical examination, the tumour showed numerous spaces which varied greatly in their size and outline, lying in a stroma of loose mesenchymal connective tissue composed of stellate and spindle shaped cells with scanty cytoplasm widely separated by abundant clear intercellular material (Figure 115). The spaces were mostly lined by flattened cells, occasionally cubical and some of them showed short stumpy papillae projecting into the lumen. Only a very occasional mitotic figure could be seen. Large areas of interstitial haemorrhage and necrosis were present in parts of the tumour.

One week after discharge from hospital, the patient noticed stiffness in the right side of her neck. This was found to be due to enlarged lymph nodes at the base of the neck. One of these was biopsied and found to be invaded by a similar tumour to the original primary. The patient kept well for a year and then she developed abdominal pain and nausea. She was re-admitted to hospital and laparotomy was again performed. The left ovary was healthy except for a warty implant on its surface. The pouch of Douglas was filled with malignant looking material and there were innumerable small studs of similar material on the peritoneum, including many on the greater omentum, but all below the level of the umbilicus. The liver, kidneys and lymphatic nodes all appeared to be in the clear and the malignancy, though inoperable, was localized to the pelvis, broad ligaments, general peritoneum, omentum and the intestine. The bulk of the mass was removed mainly for biopsy reasons and most of the omentum was amputated. The patient made a good recovery, was discharged home and was able to go about but she always had some nausea and abdominal pain. Three months later, she died.

Case 2: This patient, aged 18 years, had been the subject of sharp intermittent attacks of pain in the right side of the abdomen, for the past two weeks. Over the last 24 hours, the pain has been more severe and almost continuous. Her periods have been irregular over the past three months. She was unmarried. Laparotomy was performed and a cystic tumour of the right ovary was removed. This was the size of a 20 weeks gestation, was twisted and showed no gross evidence of spread.

On pathological examination, the tumour showed an identical picture to that described for the tumour in case 1.

The patient presented 8 months later with evidence of abdominal and pelvic recurrence. Laparotomy showed the presence of gross ascites as well as nodular growths everywhere from the pelvic organs to the liver and it was decided that it was no longer possible or safe to remove the uterus and left appendages. The abdomen was closed. An attempt was made to treat the patient with endoxan. Recurrent ascites necessitated paracentesis. She died 4 months later.

SUMMARY

Very rare types of ovarian tumours, of which only one or two cases were encountered in our series of 1734 tumours, are presented in detail. They include the following tumour types: primary mesodermal mixed tumour, malignant Brenner tumour, malignant teratoma, malignant adenofibroma and mesoblastoma. The diagnostic difficulties in these rare cases are discussed.

CHAPTER 4OVARIAN ENLARGEMENT SIMULATING TUMOUR FORMATION IN A VIRILIZED
PATIENT DURING PREGNANCY

A certain degree of ovarian enlargement is usual in normal pregnancy (Nelson and Greene 1958). Polycystic change or hyperthecosis in association with virilization may also lead to a certain degree of ovarian enlargement (Scully, 1963). The enlargement, however, is usually moderate in both cases. In this unusual case, a virilized patient had two successive pregnancies and was delivered by Caesarean section each time. On both occasions, her ovaries were found to be grossly enlarged, simulating tumour formation.

Case report: Miss B.H. was first seen at the age of 16 years. She had her first period at the age of 12 years. For the first year, her periods recurred every 28 days and lasted for 4-5 days. In the second year, the interval lengthened to 3-4 months. In the third year, she had only 2 periods and in the fourth year she had only one period, 8 months before presenting for treatment. In the past 2-3 years, she noticed that her arms and legs have become covered with hair. Hair appeared on the chin during the last year. She had had appendicectomy at the age of 8 years, and had been obese ever since. A pilonidal sinus was excised 7 months before. Intermittent pain in the left flank recurred on 5 occasions during the last 4-5 months. On examination, the patient was seen to be grossly obese and hirsute. Her voice was feminine and the breasts were well developed. Striae were present on the abdominal wall. The blood pressure was 135/75. The external genitalia were normal. The

patient was admitted for investigation. X-ray of the skull was free. Intravenous pyelography was normal. Urine examination showed occasional glycosuria. Glucose tolerance test showed a fasting level of 115 and a one hour peak of 205 mgm/100 ml. Urinary neutral 17-ketosteroids were estimated on two occasions. The first specimen (970 ml.) showed 17 mgm/100 ml. The second specimen (1030 ml.) showed 5.9 mgm/100 ml. At this stage, the diagnosis of adreno-genital syndrome with a possible tumour on the right side was considered. Peri-renal insufflation was therefore performed. On the left side, the kidney was not so well outlined but there was no downward displacement. On the evidence of these radiological findings, the chances of the patient's harbouring a tumour of the adrenal were considered to be remote. The patient was put on a reducing diet and oral cortisone therapy was tried. However, no improvement was observed.

Two years later, the patient was engaged to be married. When she was next seen, her periods had returned and she looked quite a different person. One year later, she was in labour at full-term. The vagina was found to be tough and unyielding, so much so that it was found impossible to apply forceps, and it was decided to carry out Caesarean section. The foetus, a male, 6 pounds and 10 ounces in weight, was unfortunately stillborn. Anoxia, inhalation of meconium and some pneumonic reaction were the pathological findings revealed by post-mortem examination. Inspection of the ovaries at the time of Caesarean section showed gross enlargement. The external appearance was described by the operator as a mass of sago grains in blackcurrant juice. The right ovary was resected.

Two years later, the patient attended the gynaecological out-

patient department for treatment of secondary amenorrhoea. On the basis of the previous ovarian findings, she was now diagnosed as a case of the Stein-Leventhal syndrome. Conservative management resulted in the return of normal menstruation and she was followed up for about one year. When she was next seen, about one year later, she was pregnant for 12 weeks. The pregnancy was uneventful apart from an episode of urinary infection, an attack of chicken-pox and treatment for trichomonas vaginitis. In view of her long history of infertility, it was decided to deliver her by elective lower segment Caesarean section. She was delivered of a live-born male infant, 5 pounds and 13 ounces in weight. At the time of operation, her remaining left ovary was found to be grossly enlarged to several times the normal size and again showed the sago-like appearance seen in the previous operation. A wedge was resected from this ovary.

Pathological description

The pathological picture of the ovary, on the two occasions, was essentially similar. The right ovary, removed at the time of the first Caesarean section was kidney shaped and measured 8.5 x 5.5 x 4 cms. The outer surface was studded with multiple small cysts, giving the appearance of sago grains (Figure 116). The cut surface showed multiple peripheral small cysts in the ovarian cortex (Figure 117). In the wedge resection from the left ovary, solid nodules of variable sizes were present in the cystic ovary (Figure 118).

Microscopically, marked proliferation of theca interna cells dominated the picture. Multiple layers of theca cells surrounded

growing, atretic and cystic follicles (Figure 119). The granulosa cell layer was of normal thickness or atrophic. The solid nodules seen in the gross specimen were also made up of theca lutein cells (Figure 120) and some of them were traceable to the thecal proliferation around follicular structures. Small foci of theca lutein cells were also scattered in the hyperplastic ovarian stroma (Figure 121) in both cortex and medulla. The corpus luteum was not encountered in either specimen. The superficial part of the ovarian cortex showed no marked fibrosis. The hilum of the ovary showed prominent rete tubules but no preponderance of hilus cells.

DISCUSSION

The unusual ovarian changes in this case should be discussed in relation to the virilization of the patient as well as in relation to pregnancy. That the virilization of this patient was related to the ovarian pathology is suggested by the normal findings of the investigation of the adrenal and pituitary glands as well as by the lack of response to cortisone therapy. Moreover, the ovarian pathological picture of hyperthecosis is compatible with the clinical picture of virilization. Here, it may be pointed out that there is some confusion of the literature in defining the Stein-Leventhal syndrome and the hyperthecosis syndrome. In the syndrome which carries their name, Stein and Leventhal (1935) emphasised the polycystic appearance and the thickened tunica as the outstanding pathological features. Fraenkel (1943) coined the term hyperthecosis to the condition characterised by the presence of

nests of lipid-laden lutein cells in the ovarian stroma, usually in association with virilization. It is generally recognised that both conditions do overlap. In the polycystic ovary, luteinization of the theca cells around the follicles may be prominent and foci of theca lutein cells may be seen in the stroma. An ovary, the seat of hyperthecosis, may show also peripheral cysts. In the opinion of Shippel, both conditions are facets of the same basic disturbance and should be regarded as stages of one syndrome (Shippel, 1955). Scully, on the other hand, though admitting that the two syndromes overlap in both their clinical and pathological pictures, prefers to classify them separately, since in their classical forms they can be distinguished (Scully, 1963). Thus, clinically, the presence of Cushingoid features and overt virilization characterizes hyperthecosis. In the Stein-Leventhal syndrome, virilization is usually limited to hirsutism and Cushingoid features are not common. Therapeutically, the good results of wedge resection are less frequently obtained in the hyperthecosis syndrome. Our case combines features of both conditions and affords an example of their overlap. The gross obesity, abdominal striae as well as the diabetic tendency suggest Cushingoid features of the hyperthecosis syndrome. Pathologically, though admittedly a pregnancy effect is added, multiple peripheral cysts were present together with foci of luteinized cells in the stroma. In this discussion, the term hyperthecosis will be used rather loosely to embrace both conditions.

Spontaneous pregnancy in the hyperthecosis syndrome is not unusual (Lynch et al. 1959). The influence of psyche is well demonstrated in our case. The psychological effect of the patient's engagement could override the abnormal pathology and induce the return of ovulatory menstruation.

In discussing the relation of the ovarian changes in this unusual case to pregnancy, it may be noted that normal pregnancy is usually associated with some degree of hyperplasia and luteinization of the theca cells (Nelson and Greene, 1958). The exact extent of this change is ill-defined but does not seem to be marked (Lynch et al. 1959). However, pregnancy may rarely be the cause of marked ovarian enlargement due to hyperplasia of theca-lutein cells. This enlargement may be cystic or solid in nature. Cystic enlargement is exemplified by the multiple theca-lutein cysts often associated with trophoblastic growths. It may however be encountered in their absence. Girouard et al. (1964) reviewed 15 such cases from the literature and added two cases of their own. Of the 17 cases, 6 were concomitant with foetal hydrops from rhesus sensitization, 5 were associated with multiple pregnancy and 6 were encountered with apparently normal pregnancies. The most plausible explanation for this cystic change is a sustained high level of chorionic gonadotrophin. Solid ovarian enlargement in relation to pregnancy has been recently described under the title of pregnancy luteoma (Sternberg, 1963). The pathological lesion has been interpreted as probably representing an abnormal hyperplasia of theca-lutein cells (Sternberg and Barclay, 1966). The only significant common factor in these cases was multiparity. This type of pregnancy luteoma has been, so far, never described in relation to trophoblastic neoplasms and there seems to be no evidence for incriminating the same etiologic factor, a high level of chorionic gonadotrophin, in its causation.

The exaggerated hyperplasia of theca-lutein cells in our case was of a solid nature and similar to that described in the cases of

pregnancy luteoma. The findings in our case suggest that this type of exaggerated hyperplasia of theca-lutein cells probably has as an important underlying factor, a pre-existent hyperplasia of theca-lutein cells. Shippel (1955) discussed the role of pregnancy in the production of the hyperthecosis syndrome and suggested that hyperthecosis may persist after pregnancy. It is possible that the repeated pregnancies may have a cumulative effect and this may explain the relation of pregnancy luteoma to multiparity. Shippel (1955) also pointed out that several factors may lead to hyperplasia of the theca cells. These include anovulatory cycles, conditions which tend to stimulate the reticulo-endothelial system and local pelvic irritative conditions. Undoubtedly, however, an element of ovarian responsiveness modifies the effect produced by these factors in the production of hyperthecosis and possibly also will play a role in defining the response to pregnancy.

Data from the literature about the picture of the ovary during pregnancy in patients diagnosed clinically as suffering from hyperthecosis are very scanty, although spontaneous pregnancy in these cases is not unusual. Lynch et al. (1959) described 2 such cases, which showed marked hyperplasia of theca-lutein cells.

Exaggerated hyperplasia of theca-lutein cells may be encountered in the absence of pregnancy. Cases are on record in which the ovary was grossly enlarged as to simulate a tumour. These include cases described by Geist and Gaines (1942), Fraenkel (1943), Rottino and McGrath (1943), Gemzell et al. (1956), Scully (1962), Koss et al. (1964) and Travis et al. (1965). The case reported by Stein et al. (1963) possibly also belongs to this group. The exaggerated hyperplasia in these cases may have had as a possible

underlying factor a long standing condition. In 3 of the aforementioned cases, the patients were over 40 years old and in another 6 the age was above 30 years. In the case of Stein et al. (1963) the patient was 26 years old but she had 4 pregnancies and during each of them an exaggeration of hirsutism was noted, possibly corresponding to an exaggerated hyperplasia of the theca cells.

As in other fields of ovarian endocrine pathology, it is futile here to attempt to correlate the hormonal potential with the morphological picture. The multiple theca-lutein cysts, well known in trophoblastic growths, are usually associated with no clinical evidence of abnormal hormonal activity. However, in the unusual case reported by Alexander and Beresford (1953) multiple theca-lutein cysts of a similar pathological appearance were apparently responsible for virilization of the patient. Reported cases of pregnancy luteoma were usually of no clinical endocrine significance. However, in the case of Malinak and Miller (1965), pseudo-hermaphroditism of the female foetus was observed. Culiner and Shippel (1949) described a patient who developed signs of masculinization during pregnancy and at the time of Caesarean section her ovaries were noted to be grossly enlarged. Follow up after delivery showed a return of the ovaries to normal size. Unfortunately, in this case pathological examination was not performed but apparently it belongs to the same group of cases we are discussing. These and other examples illustrate the fallacy of attributing one particular type of steroid production to a particular cell in all its physiological and pathological states. From morphological appearances, cells may be characterised as showing a picture compatible with steroid production. However, in

the present state of our knowledge, it must be admitted that there is no telling what type of steroid or steroids that would be, nor how much of it is produced.

SUMMARY

An unusual case of ovarian enlargement, simulating tumour formation, is described in a virilized patient at full term during two successive pregnancies. The enlargement was due to hyperplasia of the theca-lutein cells. The relevant literature on ovarian changes in virilized women (in the absence of tumours) and on ovarian changes during pregnancy is discussed. This condition should be borne in mind because if encountered an ovarian biopsy is all that is needed and there is no justification for more radical surgery.

P A R T I I I

CYTOGENETIC STUDIES

CHAPTER 1INTRODUCTIONSEX CHROMATIN

The demonstration of a sex difference in mammalian interphase nuclei by Barr and Bertram in 1949 opened a new era in the field of cytogenetics. Studying the effect of fatigue on the neurones of the cat, the two workers noted the movement of what appeared to be a nucleolar satellite during depletion and restoration of Nissl material. This satellite was present only in female cats. This is how this far reaching discovery of nuclear sexual dimorphism was first made. Subsequent studies extended this observation to other mammals and to man (Moore and Barr 1954). Apart from unsuitable necrotic nuclei, the imprint of sex was shown to be present throughout the various tissues and organs of man. The sex chromatin was also shown to be still recognizable in pathological, inflammatory or neoplastic tissues (Moore and Barr 1955b, 1957). In an attempt to simplify the sex chromatin test for clinical use and to avoid the minor operation of obtaining a skin biopsy specimen, the buccal smear method was introduced by Marberger et al. (1955) and Moore and Barr (1955a).

Morphological characteristics of the sex chromatin

Position: The favoured position of the sex chromatin is against the nuclear membrane. There is also a tendency for the sex chromatin to be located at one tip of elongated nuclei such as those of smooth muscle fibres (Moore and Barr 1954). It is not known why the sex chromatin tends to assume a definite position in

the nucleus. This position, however, seems to vary in various species, various tissues, conditions of growth and altered cell metabolism. The change in the position of the sex chromatin during chromatolysis of motor neurones in the cat was the observation that led to its discovery. Other possible positions for the sex chromatin apart from lying adjacent to the nuclear membrane, are a juxta-nucleolar position and a free position in the nucleoplasm.

Shape: The sex chromatin is usually planoconvex, one side being flattened against the nuclear membrane, but it may be spherical, triangular, irregular or disc shaped (Moore and Barr 1953). The shape of the sex chromatin is influenced by its position in the nucleus. When it lies free of other components of the nucleus, it tends to be spherical.

Size: The sex chromatin of female nuclei is of the same order of size in different representatives of the mammalian class, with mean dimensions of 0.8×1.1 micron but there appears to be minor differences in its size from one type of cell to another. An abnormally small or large size of the Barr body may indicate an abnormal X chromosome.

Staining affinities: The reaction of sex chromatin to stains is that of chromatin and chromocentres in general. It has an affinity for basic dyes such as haematoxylin. Deoxyribonucleic acid (DNA) is apparently an important constituent of the sex chromatin, since it is Feulgen positive. The Feulgen stain can differentiate the sex chromatin from nucleoli.

Frequency of nuclei with sex chromatin: The percentage of nuclei with sex chromatin in the female varies according to the type

of tissue examined, the type of preparation, the technical quality and the use of different criteria by different authors. The nervous tissue shows a high percentage of sex chromatin while the buccal mucosa shows a much smaller percentage. The influence of the type of preparation is shown by the difference between smears or whole mounts of thin membrane where entire nuclei are examined and histological sections where only sections of the nuclei are available. Some workers include in their counts only chromocentres adjacent to the nuclear membrane while others include chromocentres of identical morphology in other positions as well. Generally, in suitably stained material, a percentage of 50 to 80 per cent of chromatin positive nuclei (with an average of 66 per cent) is to be expected in female tissues. In similar preparations from males, a chromocentre that is larger than other chromatin particles but usually smaller than the typical sex chromatin of the female occurs in 0-15 per cent of nuclei with an average of about 5 per cent.

To explain the absence of a Barr body (sex chromatin) in certain cells and its presence in others with the same XX chromosome complement, De Mars (1962) has claimed that the sex chromatin is present only during the few hours preceding cell division, i.e. only after chromosome duplication has taken place. Miller (1964), on the other hand, suggested that the Barr body is present only when the X-chromosome forming it is condensed and is absent when the involved X is despiralized and presumably replicating its own DNA. This would account for the lower incidence of chromatin positive cells in rapidly dividing cultures from human females, since a greater proportion of these cells would be duplicating the X-chromosome that forms the sex chromatin.

A diminished incidence of Barr bodies may be due to sex chromosome mosaicism when one of the cell lines has a single X chromosome (Vaharu et al. 1961) or it may be encountered in cases with a deletion of part of one X chromosome (Jacobs et al. 1960). Thus, a low positive count may direct attention to a sex chromosome abnormality.

A possible effect of antibiotics and hormones on the sex chromatin has also been suggested. Sohval and Casselman (1961) suggested that certain antibiotics, possibly by inhibiting DNA synthesis, might reduce the size of Barr bodies and could lead to misinterpretations of sex chromatin tests. Smith et al. (1962) have shown a low incidence of sex chromatin in newborn females. A diminished incidence of Barr bodies in patients treated with hydrocortisone or A.C.T.H. has also been claimed (Taylor, 1963b). Variations in the incidence of chromatin positive cells have also been described in the different phases of the menstrual cycle (Del Campo and Ramirez, 1965).

Number of sex chromatin masses per nucleus: Usually one mass of sex chromatin is encountered in nuclei that are normally diploid. In those rare sites where the presence of polyploid nuclei is normal e.g. liver cells, amnion and bronchial epithelium, more than one mass of sex chromatin may be present. In patients with chromosomal abnormalities and in abnormal embryonic as well as malignant tissue, also more than one mass of sex chromatin may be present. In diploid cells, the maximum number of sex chromatin masses per cell can be worked out from the formula, $n - 1$ where n is the number of X chromosomes. Observations on polyploid cells suggest that one

diploid set of autosomes seems to prevent the formation of sex chromatin from one X-chromosome (Editorial, Lancet 1960). Thus, in a tetraploid cell with an XXYY pattern, sex chromatin would be absent in spite of the presence of two X chromosomes, and in a tetraploid XXXX cell two sex chromatin masses are seen instead of three (Klinger and Schwarzacher 1960). Different from the $n - 1$ rule operating in diploid cells, an $n - 2$ rule seems to hold in tetraploid cells. Triploid cells, on the other hand, seem to behave as diploid cells in this aspect (Mittwoch et al. 1963).

Derivation of the sex chromatin

Sex chromatin was originally thought to arise by fusion of heterochromatic portions of two X chromosomes (Barr and Bertram 1949). It is now evident that sex chromatin is formed from a single heterochromatic X chromosome (Miller 1964). This is supported by observations along several lines.

The direct demonstration of abnormal sex chromosome complements in individuals with abnormal sex chromatin patterns has now made it possible to show that the maximum number of sex chromatin masses in cells from any individual is one less than the number of X chromosomes (Barr and Carr 1962). The sex chromatin masses in these polysomic X individuals are all of the same size as the Barr bodies in normal females with two X chromosomes. The possible explanation is that each sex chromatin mass is formed by a single heteropyknotic X chromosome and that one X chromosome in each diploid XX cell is isopyknotic and cannot form a Barr body.

More convincing evidence of the single X nature of the sex chromatin has come from studies of the pattern of chromosome

duplication by the use of radioactive DNA precursor (H^3 - thymidine). One of the two X chromosomes of normal females completes replication of its DNA later than most of the other chromosomes of the complement (Gilbert et al. 1962). No late replicating X chromosome is present in XY males or XO females. In XXX and XXXY individuals there are two late replicating X chromosomes and in an XXXX female there are three (Grumbach et al. 1963). The number of X chromosomes with a late replication is thus identical with the maximum number of Barr bodies and one less than the number of X chromosomes. Furthermore, the late replicating X chromosomes tend to lie at the periphery of metaphase figures from colchicin-treated cultures (Mukherjee et al. 1964), a position comparable to that of sex chromatin in interphase nuclei.

Another bit of evidence of the single X origin of sex chromatin is the occurrence of very large (or very small) Barr bodies in individuals with one very large (or very small) X chromosome in addition to a structurally normal X chromosome. In all such cases tested, the structurally abnormal X chromosome has been the late replicating X chromosome which supports the hypothesis that Barr bodies are formed only by late replicating X chromosomes (Miller 1964).

Genetic evidence also points to the existence of only one single X chromosome in active state in each cell. Geneticists have known for a long time that heterochromatin i.e. chromosomal segments that stain intensely during interphase and prophase when other segments stain very lightly, is genetically inactive (Cooper 1959). The genetic inertness of heterochromatin has recently been shown to be due to its inability to synthesize messenger RNA

(Hsu, 1962). Since females possess two X chromosomes while males possess only one, a double dose for each of the genes on the X chromosome is to be expected in females. For autosomal genes, such a double dose of allelo-morphic genes is bound to produce double the gene effect. This does not happen with the X chromosomes denoting that one must be inactive. This phenomenon, called dosage compensation, can be studied in man, by measuring the activity of the enzyme glucose-6-phosphate dehydrogenase (G-6-PD) in red blood cells. The formation of this enzyme is controlled by a gene on the X chromosome. The main activity of G-6-PD in red blood cells of normal males is approximately the same as that in normal females, in XO individuals and in individuals with 3 or 4 X chromosomes (Grumbach et al. 1962).

An attractive explanation for the presence of a single active X chromosome is provided by the Lyon hypothesis (Lyon, 1962). According to this hypothesis, at a certain stage of embryonic development one of the two X chromosomes in somatic cells of the normal mammalian (including human) female is inactivated so that it takes little or no part in directing cellular activities. The inactive X chromosome becomes heterochromatic and capable of forming sex chromatin. The other X chromosome remains active. This process of inactivation of the X chromosome affects at random the maternal or paternal X chromosome. Once inactivation has taken place, the same X chromosome remains inactivated in the progeny of each cell of the embryo. The hypothesis thus implies that all the somatic cells in the body of the mammalian female can be divided into two classes: those with maternal derived (matroclinous)

active X chromosome and those with paternal derived (patroclinous) active X chromosome. It is interesting to note that this could be verified in the mule, whose two X chromosomes coming from a horse and a donkey are morphologically different. Mukherjee and Sinha (1964) have shown that the matroclinous and the patroclinous X chromosomes were equally frequently the late replicating chromosomes in leucocytes cultured from a mule.

HUMAN CHROMOSOMES

Reliable studies on human chromosomes have been possible only recently after the introduction of satisfactory methods for chromosome analysis. Classical cytological procedures, without the aid of ancillary treatment, do not allow for easy or reliable observation (Ford and Hamerton, 1956). Some means is needed to spread and disperse the densely crowded chromosomes in the equatorial plate during metaphase. This could be achieved by hypotonic treatment to cause swelling of the cytoplasm and then flattening of the mitotic cells to spread the chromosomes as far as possible into a single plane inside the cell either by direct mechanical squashing or by air drying after alcoholic fixation. It is essential also to get as much dividing cells for examination as possible. In tissues where mitotic activity is naturally marked, e.g. bone marrow and testis there is usually enough cells for examination. In other tissues, culture in vitro is needed to obtain sufficient dividing cells for the purpose of analysis. One of the steps of modern technique is also the pretreatment with drugs that inhibit the formation of the mitotic spindle such as colchicine or its less toxic derivative desacetyl methylcolchicine (Colcemid, Ciba). The exposure to a spindle inhibiting drug has

a characteristic effect on the morphological appearance of the chromosomes. The chromosomes appear shorter than they would have been at metaphase in a natural mitosis and the chromatids (the two half-chromosomes which will become daughter chromosomes at anaphase) are somewhat thicker than normal and diverge to a greater or lesser degree from the centromere (the point where they remain attached to one another). The consequence is that instead of being linear structures in which the precise position of the centromere may be difficult to define, they have a bilateral symmetry, looking something like an X when the centromere is median and like a V when it is nearly terminal.

Chromosome count: Early in 1956, Tjio and Levan working in Sweden reported counts of 46 chromosomes in human tissues. Their preparations were of high quality and they revealed the chromosomes of the diploid somatic complement with a great degree of detail. Their preparations were made from tissue cultures set up from lung explants obtained from aborted fetuses. Before the work of Tjio and Levan (1956), the diploid somatic complement in man was taken as 48.

Identification of individual chromosomes: The important features for the identification of a chromosome are its relative length and its centromere position. The particular way in which the chromatids chance to lie is irrelevant. Experience has also shown that the presence of satellites on the short arms of the chromosomes does not provide a means for discrimination between them. For the identification of individual chromosomes, the classification generally adopted is that agreed upon at a meeting in Denver by the

human chromosome study group in 1960. In the Denver system, human chromosomes are classified into seven groups, each group designated by the arabic numerals of the extreme chromosomes of the group, joined by a hyphen: group 1-3, 4-5, 6-12, 13-15, 16-18, 19-20 and 21-22. The Denver system may, however, be cumbersome to use in certain instances, for example in the expression of a reciprocal translocation between undefined members of two separate groups. In this case, the lettering system used by Patau et al. (1960) is more convenient. In this system, the 7 groups can be indicated by the letters A to G. Thus, it is easier to refer to a D-G translocation instead of saying that there is a translocation between a chromosome from group 21-22 and another from group 13-15. The Denver numbering can be combined with Patau letters whenever individual chromosomes could be unequivocally distinguishable, for example A_1 , A_2 and A_3 (Hauschka, 1961).

The characteristics of the various chromosome groups are as follows:

- Group A (1-3): These are large chromosomes with approximately median centromeres. The three chromosomes are readily distinguished from each other by size and centromere position.
- Group B (4-5): These are large chromosomes with submedian centromeres. The two chromosomes are difficult to distinguish but chromosome 4 is slightly larger.
- Group C (6-12): This is a group of medium-sized chromosomes with submedian or subterminal centromeres. The X chromosome is difficult to distinguish from pair 6.

This large group presents major difficulties in the identification of individual chromosomes. At the London conference on the normal human karyotype (1963), it was suggested that the four most metacentric pairs of chromosomes in the group should be numbered 6, 7, 8 and 11. The three submetacentric chromosomes should then be numbered 9, 10 and 12.

Group D (13-15): These are medium-sized chromosomes with nearly terminal centromeres (acrocentric chromosomes). Contrary to the belief at the time of the preparation of the Denver report, the presence of satellites on the short arms of some members of this group in occasional preparations cannot be relied upon in distinguishing individual members of the group.

Group E (16-18): These are rather short chromosomes with approximately median or submedian centromeres. Pair 16 is fairly readily separable from the group on account of its nearly median centromere.

Group F (19-20): These are two very similar short metacentric chromosomes.

Group G (21-22): These are very short acrocentric chromosomes. Again, contrary to the belief at the time of the Denver report the presence of satellites proved to be unreliable in the differentiation of the

two members of this group. The Y chromosome is sometimes difficult to separate from this group, though commonly it appears to be a little longer and to have a more nearly terminal centromere. There is some indication that it varies in size from one individual to another.

CHAPTER 2TECHNIQUES

The techniques used in our study and requiring special description are:

1. The buccal smear method for the study of sex chromatin.
2. The Feulgen reaction for demonstration of sex chromatin.
3. The method of obtaining chromosome preparations from cultures of peripheral blood.
4. The method of obtaining chromosome preparations from cultures of the ovarian teratoma.

The buccal smear method for the study of sex chromatin:

The smear is obtained in the following way: The mouth is opened and the angle drawn laterally with the little finger of the hand holding a clean slide. This reveals the buccal pouch. Gently but firmly a tongue blade is drawn forward a number of times along the buccal reflex or floor of this pouch. A white milky material is obtained which is rich in cells. While the slide is held over a bottle of fixative, this milky material is quickly spread upon the glass slide and immediately dropped into fixative (95 per cent ethyl alcohol). The Feulgen method was used in staining in our cases.

The Feulgen method of staining (Pearse 1960):

1. Bring sections to water and remove mercury if necessary.
2. Rinse briefly in cold N-HCl.
3. Place in N-HCl at 60°C for the optimum time for hydrolysis (5 minutes for material fixed in Zenker).

4. Rinse briefly in cold N-HCl. and then in distilled water.
5. Transfer to Schiff's solution for the optimum time ($\frac{1}{2}$ to 1 hour with de Tomasi).
6. Drain and rinse in three changes of freshly prepared bisulphite solution (5 ml. 10 per cent $K_2S_2O_5$, 5 ml. N-HCl., water to 100 ml.).
7. Rinse in water.
8. Counterstain if desired (1 per cent aqueous light green for 1 minute or 0.5 per cent alcoholic fast green for $\frac{1}{2}$ to 1 minute).
9. Dehydrate in alcohol.
10. Clear in xylene and mount in canada balsam.

Result: DNA appears in shades of reddish purple.

Method of obtaining chromosome preparations from cultures of peripheral blood:

The method used is based on that described by Moorhead et al. (1960). The steps involved are:

1. setting up the culture.
2. cytological processing.
3. preparation of the slides.

1. Setting up the culture:

(All containers, instruments and solutions are sterile)

1. Inject 15 - 20 ml. freshly drawn blood into a universal container containing commercial heparin.
2. Add 0.4 ml. phytohaemagglutinin, leave in iced water for 30 minutes and then centrifuge gently (350 r.p.m.) for 5 - 10 minutes. By this time the red cells have sedimented and

the majority of the nucleated cells are in suspension in the plasma.

3. With a syringe and long needle transfer the plasma containing the cells into a clean universal container.
4. Mix by aspiration and do a nucleated cell count.
5. The cell count is adjusted to between 1,000 and 2,000 cells per cubic millimeter by the addition of tissue culture medium 199. Usually, the final suspension has 1 part plasma: 4 parts 199.
6. Six to 10 ml. aliquots of the final suspension are set up in universal containers.
7. The cultures are incubated at 37°C for approximately 2 to 3 days.

2. Cytological processing:

1. Three hours before harvesting the culture, 0.1 ml. of 0.02 per cent solution of colcemid is added for each ml. of culture.
2. After the colcemid has been in the culture for about 3 hours, the culture is well shaken and transferred to a 15 ml. centrifuge tube. It is then spun down and the supernatant fluid removed leaving only one drop in which the cells are resuspended.
3. The cells are resuspended in 5 - 10 ml. of 0.95 per cent solution of sodium citrate previously warmed to 37°C and left in this solution for 15 to 30 minutes.
4. The cells are spun down and the sodium citrate removed except for one drop in which the cells are resuspended. Two ml. of fixative, consisting of 3 parts ethyl alcohol:1 part glacial acetic acid, is added to the cells very slowly and carefully, with constant agitation of the cells.

5. The cells can be stored in this fixative in the refrigerator.

3. Preparation of slides:

1. The cells are spun down from fixative immediately prior to making the slides. The fixative is removed and the cells are resuspended in fresh fixative consisting of 3 parts methyl alcohol:1 part glacial acetic acid.
2. The cells are again spun down, the fixative removed and they are resuspended in fresh fixative consisting of 3 parts methyl alcohol:1 part glacial acetic acid.
3. A clean slide is immersed in iced water till it is cold, the excess water is shaken off and a couple of drops of the cell suspension are dropped on the slide. The majority of cells immediately stick to the slide which is then quickly tilted on filter paper and the excess liquid drawn off. The slide is immediately waved vigorously over a spirit lamp until completely dry. This usually takes about 20 seconds. (A hair dryer can also be used for the same purpose).
4. The slide is stained in 1 per cent solution of aceto-orcein for 20-60 minutes, rinsed quickly in 45 per cent acetic acid, dehydrated, cleared and mounted.

Solutions

1. Heparin - Evans Pulvarin Heparin.
2. Phytohaemagglutinin - Burroughs Wellcome Phytohaemagglutinin, diluted as suggested by the manufacturers.
3. Tissue culture medium 199 Glaxo Laboratories.
Eagle's medium is equally satisfactory.
4. Colcemid - Ciba Laboratories.

5. Acetic Orcein - 1 gm. synthetic orcein added to 60 ml. hot glacial acetic acid, cooled, then 40 ml. distilled water added and then filtered.

Method of obtaining chromosome preparations from cultures of the ovarian teratoma

The technique used was based on that described by Harnden (1960) for obtaining chromosome preparations from skin biopsy. The steps involved are:

1. Setting up the culture.
2. Maintaining the culture.
3. Cytological processing.
4. Preparation of slides.

1. Setting up the culture:

The biopsy material is cut into pieces of approximately 1 mm. square and placed in the bottom of the culture bottle (baby feeding bottle). The pieces of tissue are embedded in a plasma clot by adding equal amounts of chick embryo extract and cock plasma. Culture fluid is added. The bottle is gased with 5 per cent CO_2 in O_2 , sealed and kept at 37°C .

2. Maintaining the culture:

1. The primary culture is left untouched for up to 5 days by which time cells should have started to emerge from the explants.
2. The medium is now changed three days a week throughout the life of the culture.
3. After 10 days or a fortnight cells should have spread extensively from the explants. The medium is removed and replaced by pre-warmed 0.25 per cent trypsin in Ca and Mg free Hanks' solution. The culture is then incubated for about 10 minutes at 37°C by

which time the cells should have detached from the bottle. The fluid containing the cells is collected and centrifuged very gently for 5 minutes and the pellet resuspended in culture fluid.

4. This suspension is transferred to a culture bottle with an appropriate amount of culture fluid (10 ml. to an 8 oz. baby feeding bottle). The bottle is gased with 5 per cent CO_2 in air and sealed with a silicone rubber bung and incubated.
5. After a few days the bottom of the bottle is covered with a monolayer of cells. This culture is trypsinized and routine subcultures made from it.

3. Cytological processing:

1. Select a culture showing numerous mitotic figures; a moderately heavy culture about 24 hours after subculture is usually suitable.
2. Colchicine to a final concentration of 0.00025 w/v is added without interrupting incubation and allowed to act for about 4 hours.
3. The cells are then digested and collected as for a subculture but they are resuspended in Hanks basic salt solution. They are immediately spun again and resuspended in 0.95 per cent sodium citrate and the hypotonic treatment continued for about 20 minutes.
4. The cells are then spun down at 500 r.p.m. for 5 minutes and the supernatant decanted.
5. The cells are flicked up in the remaining drop of liquid and fixative (3 parts absolute alcohol:1 part glacial acetic acid) added very slowly. The cells are left to fix for at least 30 minutes.

4. Preparation of slides:

The fixative is replaced by 75 per cent acetic acid and one or two drops of the suspension are placed on a clean slide which has been standing in iced water (the surplus water has been shaken off) and the slide is immediately dried. The slide is stained in 2 per cent acetic orcein for at least 3 hours at 37°C, rinsed quickly in 45 per cent acetic acid, dehydrated, cleared and mounted.

The culture fluid

This consists of:

70 per cent Eagle medium (Burroughs Wellcome)

20 per cent human serum

10 per cent chick embryo extract.

The pH of the medium is adjusted to 7.2 by means of bicarbonate which equilibrates with the CO₂ in the gas phase. Kanamycin is routinely used as an antibiotic. Penicillin and cephalosporin are kept in reserve.

CHAPTER 3SEX CHROMATIN AND CHROMOSOME ANALYSIS IN OVARIAN TERATOMAS

The development of ideas about the nature and origin of teratomas provides an interesting reflection of the rate of progress in methods of scientific investigation. Theories of teratogenesis may be seen to have followed four phases.

The striking foetus-like characteristics of the teratoma have been known for centuries and these encouraged the belief that they were the product of conception in the form of an included twin or an ovarian pregnancy or as the outcome of parthenogenesis (Blackwell et al. 1946). These speculations were based on simple observations of the gross morphology of teratomas.

Later, the application of rigorous histological techniques to these specimens quickly exposed the inaccuracies and exaggerations of earlier descriptions and discredited the idea of the foetal nature of these tumours (Nicholson 1930). The alternative suggestion was developed that teratomas are neoplasms originating from foci of cells which, at a very early stage of embryogenesis, escape from the control of primary organizers (Willis 1960). These cells retain their primitive totipotency and may produce tumours which contain tissues representative of all three germ layers, but they totally lack any vertebral organization.

The recent demonstration of sex chromatin (Barr and Bertram, 1949) provided one method of testing these conflicting hypotheses. The concept of the teratoma as a neoplasm implied that it should have the same genetic sex as the host, whereas older ideas of the foetal nature of the teratoma suggested that there might be some

discrepancy between the sex of the host and the tumour. The first report of sex chromatin studies on teratomas revealed such a discrepancy in that some testicular teratomas proved to be chromatin positive (Hunter and Lennox 1954). Since then, a large number of similar studies on both female and male teratomas have been reported (Tavares, 1955; Moore and Barr, 1955b; Levi, 1955; Sohval and Gaines, 1955; Mancini, 1956; Brünning, 1956; Riviere, 1956; Tavares, 1958; Maltzeff and Sacco, 1958; Myers, 1959; Hienz, 1960; Theiss *et al.* 1960; Taylor, 1963a; Girelli and Rigoli, 1963; Talwalker, 1963; Taylor, 1965). Undoubtedly, sex chromatin gives invaluable information about the X chromosome complement of the cell. However, it is less reliable in malignant tissues (Lennox, 1963); it is subject to technical misinterpretations (Lennox, 1963); it gives no idea of the Y chromosome complement of the cell; and it gives no information about autosomes.

Consequently, it is now necessary to supplement sex chromatin studies with full chromosome analysis. So far, only three reports describing a total of 8 teratomas have been described (Galton and Benirschke, 1959; Corfman and Richart, 1964; Harnden, 1965). We have studied the sex chromatin pattern in 187 ovarian teratomas. In 6 other cases, chromosome analysis was also performed.

Material and Methods

The material for the sex chromatin study was selected from the material collected in the department over the past 10 years. One hundred and eighty seven specimens were considered adequate for study. All of these were benign ovarian cystic teratomas with the single exception of one solid histologically benign teratoma. The

specimens had been fixed in formalin or Zenker fluid and the slides were stained with haematoxylin and eosin. In a few cases, additional sections were cut and stained with the Feulgen method. Various tissues were studied in each specimen but squamous epithelial cells were selected for routine counting, 100 suitable nuclei being studied in each case.

Six fresh benign cystic teratomas were subjected to chromosome analysis, according to the method previously outlined. From each specimen, several representative pieces of tissue were taken. Each was divided into two parts, one for histologic study and the other for tissue culture, in order to allow accurate correlation between the nature of the tissue and its chromosome constitution. The small fragments of tissue were grown as explants in plasma clots and chromosomes were prepared from subcultures.

Results

Sex chromatin: In the 187 specimens we studied, the percentage of cells with demonstrable sex chromatin varied between 23 and 87 per cent with a mean of 56 per cent. The sex chromatin was well demonstrable in almost all tissues of the teratoma (Figs. 122, 123, 124, 125). In several tumours, an occasional cell appeared to show double sex chromatin, but in no case was this the predominant cell type. These findings indicate that our specimens were chromatin positive as in normal female tissues.

Chromosome analysis: The distribution of chromosome counts is shown in Table XIV.

TABLE XIV

Distribution of chromosome counts in female benign cystic teratomas

Case No.	Total	Less than 44	44	45	46	47	48	More than 48
194	30	-	3	1	26	-	-	-
195	37	-	-	-	34	1	2	-
246	24	3	1	1	18	-	-	1
251	4	-	-	-	4	-	-	-
259	17	1	1	1	11	1	1	1
280	42	5	2	4	30	-	-	1
TOTAL	154	9	7	7	123	2	3	3

As is shown in the Table, a total of 154 cells were counted, of which 123 had 46 chromosomes, 23 had counts in the hypoploid range and 8 cells were hyperploid. Endoreduplication was noticed in one cell from case no.259. Visual analysis revealed a normal karyotype in all 6 cases (Fig. 126). Case no. 280 showed a minor structural variation, two members of the D group having marked enlargement of the short arms. This is unlikely to be of significance and is regarded as a normal variant (Court-Brown et al. 1965). All of these 6 cases, thus, show a normal female, XX sex chromosome complement.

DISCUSSION

The results of previous studies of sex chromatin in ovarian teratomas, together with our own data, are summarized in Table XV. The vast majority are chromatin positive, but a few have been reported as chromatin negative. Undoubtedly, some of these anomalous results were derived from material which was unsuitable for the demonstration of sex chromatin. Furthermore, in any highly malignant tissue there may be anomalous sex chromatin and this may explain some of the chromatin-negative malignant ovarian teratomas (Lennox, 1963). There is also one well-documented benign ovarian teratoma which contained double sex chromatin (Taylor, 1962). Similar results from studies on extra-gonadal teratomas in females have been reviewed by Taylor (1963a), and comparable findings of chromatin negative and doubly chromatin positive cells have been reported. It seems likely that whereas the majority of female teratomas have a "female" sex chromatin pattern, a few may show anomalous nuclear sex.

The results of sex chromatin studies in male teratomas, both testicular and extragonadal, have been reviewed by Ashley (1962) and Taylor (1963a). Here, the findings are even more striking in so far as 40 per cent are chromatin positive, while a few cases of sex chromatin mosaicism and double sex chromatin have also been recorded. Many testicular teratomas are highly malignant and this may invalidate the interpretation of some of these results. Nevertheless, anomalous sex chromatin has been clearly demonstrated in benign male teratomas and it is impossible to dismiss all of these results as artifacts.

These findings suggest that there may be some discrepancy between the nuclear sex of the host and that of the teratoma, and so cast doubt on the concept of the simple neoplastic origin of these tumours and raise again the possibility of their origin from germ cells. One simple explanation would be that these anomalous tumours may arise in hosts with similarly abnormal sex chromatin. Teratomas have been rarely described in males with Klinefelter's syndrome (Taylor, 1963a), but it has been firmly established that the majority of teratomas occur in hosts with normal sex chromatin (Myers, 1959; Ashley and Theiss, 1958; Ashley and Mostofi, 1959; Court-Brown et al. 1960).

The most plausible theories of teratogenesis from germ cells are based on hypothetical mechanisms of parthenogenesis.

1. Normal haploid gametes may fuse to form diploid cell (Hunter and Lennox, 1954). In the female, the fusion of haploid, 23X, cells would invariably produce chromatin positive diploid cells, 46XX. In the male, the presence of both X-bearing, 23X, and Y-bearing, 23Y, cells could combine to form diploid cells with XX, XY, or YY sex chromosome complement in the ratio of 1:2:1. If YY cells are non-viable, this ratio becomes XX:XY - 1:2.
2. Reduplication of haploid gametes could also produce diploid cells (Tavares, 1955). In the female, this would invariably produce chromatin-positive cells, 46XX. In the male, it would produce equal numbers of chromatin-positive cells, 46XX, and chromatin-negative cells, 46YY. This theory implies that cells with a YY sex chromosome complement are viable. Data derived from the ratio of chromatin-positive to chromatin-negative male teratomas are still inconclusive in favouring either of these

hypotheses (Taylor, 1965).

3. Meiotic nondisjunction may be followed by the fusion of aneuploid gametes (Taylor, 1962). By such a mechanism, chromatin-negative teratomas may have a $45/XO$ chromosome constitution, chromatin-positive male teratomas may have $47/KXY$, and doubly chromatin-positive teratomas may have $47/KXX$.

Other mechanisms such as failure of reduction division or polyploidy are also possibilities. There are undoubted difficulties to all of these parthenogenetic theories. The most serious problem is that meiosis is thought to occur in males only after puberty (Hamilton, et al. 1962), while the first meiotic division in oogenesis occurs during intra-uterine development but is arrested at the dictyotene stage (Ohno et al. 1962).

It is clear that these hypothetical suggestions can be elucidated only by full chromosome analysis. So far, Galton and Benirschke (1959) have studied one malignant ovarian teratoma, Harnden (1965) has analysed one benign solid teratoma, and Corfman and Richart (1964) have studied six benign ovarian cystic teratomas. Our own report adds a further 6 benign tumours. All of these have proved to have a normal diploid female karyotype, $46/XX$.

This evidence excludes the possibility that teratomas may themselves be haploid. Furthermore, they have a normal karyotype and do not have any autosomal anomaly. This latter possibility may be suggested by the finding of an extra autosome in an amorphous acardiac foetus (Rashad and Kerr, 1965). The features of disordered organogenesis in this foetus were very similar to those of some teratomas.

These studies do not provide an answer to the origin of

anomalous sex chromatin in some teratomas since all our specimens were normally chromatin positive. Further information is more likely to come from chromosome analysis of testicular teratomas. The study of chromatin-positive male tumours should give definite evidence as to the histogenesis of teratomas.

SUMMARY

Nuclear sex was studied in 187 cases of benign ovarian teratoma and chromosome analysis was performed in 6 cases after culture. All cases studied were chromatin positive and the chromosome analysis in the 6 cases showed a 46/XX pattern. The bearing of these results on the problem of the histogenesis of the teratomas is discussed.

TABLE XV

Sex chromatin studies in female gonadal teratomas

Author	No. of cases	chromatin positive	double sex chromatin	chromatin negative	doubtful
Hunter and Lennox (1954)	10	10			
Tavares (1955)	32	32			
Moore and Barr (1955)	4	4			
Levij (1955)	20	20			
Sohval and Gaines (1955)	10	5		5	
Mancini (1956)	12	12			
Brüning (1956)	50	21		9	20
Riviere (1956)	64	64			
Maltzeff and Sacco (1958)	4	4			
Myers (1959)	47	47			
Hienz (1960)	24	17		4	3
Theiss, Ashley and Mostofi (1960)	20	20			
Taylor (1963a)	12	10	1	1	
Girelli and Rigoli (1963)	12	10		2	
Talwalkar (1963)	12	12			
Present investigation	187	187			
TOTAL	520	475	1	21	23

CHAPTER 4THE RELATIONSHIP BETWEEN OVARIAN TUMOURS AND INTERSEX STATES
WITH SPECIAL REFERENCE TO THE DISGERMINOMA AND ARRHENOBLASTOMA

The association between intersex states and ovarian tumours, particularly the disgerminoma and arrhenoblastoma, has at least two points of interest. It has immediate relevance to our understanding of the nature and histogenesis of these tumours, and it is of practical importance to the clinician in his management of intersex states.

In the first three decades of this century many clinical and pathological studies of this problem were reported and this epoch may be said to have ended with a more or less general acceptance of Robert Meyer's views. In a series of publications (1931a, 1931b), he drew a sharp distinction between two types of ovarian tumour which are associated with intersex states. In one variety, which he called disgerminoma, he postulated that the intersex state favoured the occurrence of the tumour, but the tumour was in no way responsible for the abnormal sex development. The other type he called arrhenoblastoma and suggested that this tumour was itself directly responsible for virilization so that the intersex state was reversible when the tumour was removed.

Meyer wrote disgerminoma with the prefix "dis". Most later authors have used the spelling with the prefix "dys", implying that the germinal cells from which the tumour arises have undergone an error of development or differentiation. For historical reasons we have preferred to use Meyer's original spelling.

The introduction of methods of analysis of chromosomes and sex chromatin has led to a better understanding of intersex states

and this new information has raised several problems which appear to complicate Meyer's concepts. Morris (1953) suggested that some tumours which had been previously reported as arrhenoblastomas may in fact have been testicular tumours occurring in association with the syndrome of testicular feminization in genetic males. Carpentier et al. (1956) went further and postulated that some other ovarian tumours, particularly the disgerminoma and choriocarcinoma, may also be derived from testicular tissue. Overzier (1963) considered that an intersex state should be suspected in every patient with a disgerminoma. Scott (1965) has gone so far as to suggest that an intersex state may underlie the development of all disgerminomas in the female, and he advocates a return to the term used by early French pathologists, seminoma ovarii. He implies that the testicular seminoma and the ovarian disgerminoma are identical and postulates that the presence of either of these tumours is dependent on the presence of an XY cell line in the gonad.

It seems timely to attempt a synthesis of both the cytogenetic and pathological evidence on this problem. In this chapter, we describe the results of a combined cytogenetic and histological study of 5 cases of disgerminoma and 5 cases of arrhenoblastoma, and present a critical analysis of the relevant literature.

Material and Methods

The material for this study consists of all the cases of these rare tumour types that were encountered in our files during the period of 10 years covered by our general review. Three other cases of disgerminoma were included in this study: one more recent

case, and two cases from the files of the pathology department of the Royal Hospital for Sick Children, Edinburgh (by the kind permission of Dr. A.D. Bain). The histopathology of the original slides was re-assessed. Sex chromatin was studied in slides stained both with haematoxylin and with Feulgen. The clinical records of each patient were reviewed, and whenever possible the patient was recalled for assessment of sex chromatin from a buccal smear, and for chromosome analysis of peripheral blood leucocytes.

Case reports and results

Case 1: The patient was 59 years old. She married at the age of 46 and had no children. Her periods were regular until the menopause at 52 years. Seven years later, she presented with postmenopausal bleeding. A pelvic tumour was detected, and total hysterectomy and bilateral salpingo-oophorectomy were performed. Fifteen months later, she died with a recurrent abdominal mass.

The left ovary was replaced by a disgerminoma and dermoid cyst, and the uterus contained multiple fibroids. Normal ovarian tissue could not be found in the sections made from the tumour, but the other ovary was normal. The squamous epithelium of the dermoid cyst, the healthy ovary and the uterine fibromuscular tissue proved to be chromatin positive. Some of the cells of the disgerminoma appeared to be chromatin positive but in general these nuclei were unsuitable for analysis. However, the fibroblasts of the stroma of the tumour were definitely chromatin positive.

Case 2: The patient was 16 years old and unmarried. Her periods began at the age of 15 and were subsequently irregular. Examination revealed a large pelvic tumour, and the urine showed a

high titre of chorionic gonadotrophin. At first, she was thought to be pregnant but a laparotomy was subsequently performed. A large right ovarian tumour was found, and a total hysterectomy and bilateral salpingo-oophorectomy performed. The clinical and endocrinological aspects of this unusual case have already been reported (Hobson and Baird, 1966).

The ovarian tumour was a dysgerminoma, and a corpus luteum was found in the right ovary immediately adjacent to the tumour cells (Fig. 127). The other ovary was normal and the endometrium was in the secretory phase. The healthy ovary, myometrium and tumour stroma were chromatin positive. (Fig. 128). The tumour cells were unsuitable for the demonstration of sex chromatin. A buccal smear was chromatin positive, and chromosome analysis of peripheral blood leucocytes revealed a normal female karyotype, 46/XX (Fig. 129).

Case 3: The patient, who was 18 years old and single, presented with abdominal distension, hypertension, proteinuria and pretibial oedema. At first she was thought to be pregnant with pre-eclamptic toxæmia, but pelvic examination revealed a swelling discrete from the uterus. Subsequent laparotomy showed a tumour of the left ovary and left oophorectomy was carried out.

The ovarian tumour was a dysgerminoma (Fig. 130). Nuclei of the tubal epithelium and of stromal fibroblasts of the tumour were chromatin positive (Fig. 131), but the tumour cells were unsuitable for analysis. A buccal smear was chromatin positive, and chromosome analysis of peripheral blood leucocytes revealed a normal female karyotype, 46/XX (Fig. 132).

Case 4: The patient was 19 years old and unmarried. She had a laparotomy at the age of 7 when she presented with an abdominal tumour. The right ovary contained a large tumour, and right oophorectomy was followed by a course of radiotherapy. At the age of 19 years she was treated for an incomplete abortion.

The ovarian tumour was a dysgerminoma (Fig. 133). Although the tumour cells were unsuitable for analysis, the stromal elements were chromatin positive. She had a chromatin positive buccal smear, and chromosome analysis showed a normal female karyotype, 46/XX (Fig. 134).

Case 5: The patient was 4 years old and presented with a symptomless abdominal swelling. At laparotomy a left ovarian tumour was discovered and the left ovary was removed.

This tumour was a dysgerminoma with marked lymphocytic reaction. The tumour cells were unsuitable for analysis, but stromal elements were clearly chromatin positive. This patient could not be traced for further cytogenetic investigation.

Case 6: The patient was 16 years old and presented with primary amenorrhoea. There was normal breast development, but infantile external genitalia, and neither axillary nor pubic hair. At laparotomy, there was no uterus and the gonads were slightly enlarged and lay on the brim of the pelvis. Both gonads were removed.

The gonads were at first reported to contain bilateral well-differentiated arrhenoblastomas, of testicular tubular adenoma type (Fig. 135). The capsules of the tumours resembled ovarian stroma but contained no follicles. The tubular elements of the tumour

were lined with Sertoli cells but there were no spermatogenic elements. There were occasional collections of Leydig cells. The nuclei of the stroma cells, Sertoli cells and Leydig cells were chromatin negative.

Prior to our investigation, this patient had already been found to have a chromatin negative buccal smear and a normal male karyotype, 46/XX (MacGregor, 1966). This is an illustration of the association of a testicular tubular adenoma with the clinical syndrome of testicular feminization in a genetic male.

Case 7: The patient was 18 years old and presented with primary amenorrhoea. At subsequent laparotomy, the uterus was absent and gonads were found to be enlarged. Both gonads were removed.

Again, these gonadal tumours were at first thought to be well-differentiated arrhenoblastomas and the histological picture was identical to that described in case 6. The nuclei of both the tumour cells and stromal cells were chromatin negative.

Nuclear sexing and chromosome analysis had been performed prior to our investigation, and the patient proved to be chromatin negative with a normal male karyotype, 46/XY, (MacGregor, 1966). This case represents another example of the association between testicular tubular adenoma and testicular feminization.

Case 8: The patient was 24 years old. She had been married for four years and had one child aged one year. She presented with secondary amenorrhoea and hirsutism following delivery. At laparotomy she was found to have a left ovarian tumour and this was removed. Following this operation her periods returned, the hirsutism disappeared, and she subsequently had two further pregnancies.

This tumour was an arrhenoblastoma of intermediate to sarcomatous type (Fig. 55). The Sertoli cells, Leydig cells and stromal elements of the tumour were chromatin positive (Fig. 136). The patient could not be traced for further examination.

Case 9: The patient was 14 years old and presented with abdominal pain and swelling. There was no evidence of virilization and she had not reached the menarche. At laparotomy, a large cystic left ovarian tumour was removed. She died with a pelvic recurrence 21 months later.

The tumour was an arrhenoblastoma. Nuclei of the stromal elements of the tumour were chromatin positive but nuclei of the tumour cells were unsuitable for analysis.

Case 10: The patient who was married and aged 24, presented with abdominal swelling of recent onset and secondary oligomenorrhoea for four years. At laparotomy, a large right ovarian tumour was removed.

The tumour was a well-differentiated arrhenoblastoma. The histological picture showed a predominantly tubular pattern with a few areas similar to granulosa cell growth. Nuclei of both tumour cells and stromal fibroblasts proved to be chromatin positive. This patient could not be traced for further investigation.

In summary, all five patients with a disgerminoma were phenotypically normal and had a normal female sex chromatin pattern, while three of them had a normal female karyotype. Of the five tumours originally diagnosed as arrhenoblastomas, two proved to be testicular tubular adenomas replacing the undescended testes of genetic males with the testicular feminization syndrome.

DISCUSSION

For the purpose of clarity, the discussion will be divided into 3 parts:

1. Gonadal tumours in intersex patients; a general survey.
2. Disgerminoma and intersex states
3. Arrhenoblastoma and intersex states.

1. GONADAL TUMOURS IN INTERSEX PATIENTS: A GENERAL SURVEY

The realization that the gonads of intersex patients may be the seat of tumour formation is comparatively recent. According to Gilbert (1942), the first case describing this association was that of Gruber (1859). Gilbert (1942) reviewed the literature and reported on 61 cases, including one of his own. Melicow and Uson (1959) judged the number of cases in the literature to be about 140 but they apparently did not take into consideration the overlap between the various series mentioned in their review.

Many of the reported cases, however, do not lend themselves to proper analysis. The replacement or destruction of the gonadal tissue by the tumour, especially if it is malignant, may render the diagnosis of the gonadal sex difficult or impossible. This is especially so if the tumour is bilateral and replacing both gonads or if it is unilateral but the opposite gonad is absent or rudimentary. The introduction of the methods of nuclear sexing and chromosome analysis in man has proved a great aid in these cases. A brief review of the occurrence of gonadal tumours in the various types of intersex will now be given.

True hermaphrodites:

Tumours of both testicular and ovarian types have been encountered in the bisexual gonads of true hermaphrodites. Tumours have been described in the ovotestis (Essenberg and Feinberg, 1937; Botella-Llusia, 1960), in the testis (Stirling, 1959) and in the ovary (Gresham and Fairgrieve, 1960; Bani, 1956; Weed et al. 1947). It is possible that tumours in true hermaphrodites are more frequent than what may be gathered from the case reports. The histological direct evidence of the bisexual nature of the gonads may be lost when a tumour replaces most of the gonad. Indirect evidence for true hermaphroditism may be suggested by the presence of an ovarian tumour of a type not encountered in the testis, in association with testicular tissue in the same or opposite side, even if no definite ovarian tissue could be demonstrated (Vaughn and Gonzales-Angulo, 1961). Overzier (1963) also pointed out another possible indirect evidence in these cases, if only one type of gonadal tissue is present. This is to demonstrate the opposite nuclear sex in the presence of mature testicular or ovarian tissue, cases of Klinefelter's syndrome being excluded.

Male pseudohermaphrodites:

A main feature of male pseudohermaphroditism is cryptorchidism. The liability of the undescended testes to neoplastic change has been documented by various studies (Gilbert and Hamilton, 1940; Campbell, 1959). Recent figures from the testicular tumour panel show that 6.6 per cent of patients with tumours of the testis either exhibited or gave a history of delayed testicular descent

(Fergusson, 1962). It is also recognized that the abdominal testis carries more risk of malignancy than the inguinal testis (Campbell, 1959). The exact underlying factor for this peculiar predisposition to neoplasia in the undescended testes is not known. It may be accounted for by the abnormal ectopic location of the testis where it is exposed to small unnoticed traumatic disturbances, either by muscle movement or direct violence or by the loss of the important heat-regulating mechanism of the scrotum (Murray and Ewert, 1947). It has also been suggested that a common factor may underlie both the testicular maldescent and the predisposition to neoplasia. Willis (1960) has suggested that the occurrence of the neoplasm in the retained testis has perhaps an endocrine background and that ectopia is only one expression of the gonadal anomaly or of a more general endocrine anomaly predisposing to tumour formation in the gonadal tissues. Sohval (1956) suggested a defect of gonadal development, testicular dysgenesis, to account for the liability to neoplasia in these cases. The tumour commonly found in the ectopic testes of male pseudohermaphrodites is the benign testicular tubular adenoma first noticed by Pick in 1905. This tumour is often encountered as an accidental finding, sometimes microscopic, in the ectopic testis, but it may replace the whole testis and may reach a large size (Neubecker and Theiss, 1962). The adenoma is formed of tubules lined by Sertoli cells. In the opinion of several authors, these Sertoli cell adenomas are not neoplasms in the ordinary sense but rather simple hyperplasias or hamartomas (Stalker and Hendry, 1954; Langley, 1954; Craig et al. 1961; Neubecker and Theiss, 1962). Their uniformity, marked differentiation and occasional multiplicity in the same testis support this interpretation.

Sertoli cell adenomas are very rarely encountered in the otherwise normal testis. Of the 17 cases reviewed by Stalker and Hendry in 1954, 11 were in pseudohermaphrodites and 6 in the ectopic testes in otherwise normal persons. Adlington and Salm (1960) reported bilateral tubular adenomata in the scrotal testes of a mentally retarded and enuretic baby.

The second type of benign tumour that may be encountered in the testes of these patients is an interstitial cell adenoma or Leydig cell tumour (Daino et al. 1963). Hyperplasia of Leydig cells is usually apparent in undescended testes and sometimes is so marked, or localized as to suggest an adenoma.

Malignant testis tumours of all types have been found in these cases. The commonest is the seminoma but tumours described as embryonal carcinoma (Gilbert, 1942), terato-carcinoma (Taub, 1954), malignant teratoma (Carmichael and Oldfield, 1934) and chorion-epithelioma (Ruffalo, 1953) have all been encountered. A mixture of tumour types may also be encountered (Taub, 1954).

Testicular feminization syndrome

The peculiar type of male pseudohermaphroditism associated with complete external feminization deserves special discussion. The term testicular feminization was given to this condition by Morris in 1953. Morris also noted the liability of these patients to develop testicular tumours. In a review of 82 cases, he found 7 with malignant tumours and 23 with benign testicular adenomas. Because of the risk of malignant change, Morris urged the view of prophylactic removal of the gonads in these patients. Hauser (1963), however, pointed out that the risk of malignant change,

estimated from the collected reviews of reported cases, is somewhat exaggerated because in the past this was a common type of clinical presentation. The diagnosis of the syndrome was seldom made in patients presenting with primary amenorrhoea and was only made if operative exploration was required for some complication. Nowadays, fewer cases are encountered presenting primarily with a tumour. Morris and Mahesh (1963) noted that of fifty reported cases, 30 years of age or older, eleven had malignant tumours. This, however, may not really represent a high incidence of malignancy above the age of 30, as the authors interpreted, but rather that 11 out of 50 cases who present with testicular feminization after the age of 30, do so because of the presence of a malignant tumour. This is possibly partly due to the fact that fewer and fewer patients present with conditions such as primary amenorrhoea and sterility after the age of 30 years. A comforting observation, in this connexion, is that no malignant tumour has been reported in these cases below the age of 14 years. These patients, therefore, should undoubtedly be left to complete their puberty before castration is considered.

A curious feature of the testes in this syndrome which is very rarely encountered in other types of cryptorchidism is their liability to cyst formation. These cysts may be large enough to be an indication for operation (Morris and Mahesh, 1963).

Female pseudohermaphroditism

The ovaries of female pseudohermaphrodites are rarely the seat of tumour formation. The alleged relation of the ovarian dysgerminoma to intersex states will be dealt with more fully in

another part of this discussion. Claret and McIntosh (1954) reported a dermoid cyst in the ovary of a female pseudohermaphrodite. An interesting case of endometriosis in a female pseudohermaphrodite reared as a male and presenting unusually as renal colic was reported by Zielinski and Szkodny (1964). Case 1 of Melicow and Uson (1959), in which the patient was interpreted as a female pseudohermaphrodite, is doubtful. Corpora albicantia and granulosa cysts were described but no follicles, the histological type of the tumour (a teratocarcinoma) is unusual in the ovary, the masculinization of the patient suggests the presence of functioning testicular tissue, the opposite gonad was absent and the result of chromatin sexing in the patient was stated to be doubtful.

Adrenogenital syndrome.

To my knowledge, there have been no reports of tumour formation in the ovaries of patients with the adrenogenital syndrome. Hilar cell proliferation was, however, noted in some cases (Landing, 1954; Hooft, et al. 1956). It may be difficult to distinguish these from nodules of aberrant adrenal tissue. It is interesting to note that adrenogenitalism in males may also be associated with hyperplastic testicular adrenal rests which may be difficult to distinguish from a testicular interstitial cell tumour (Glenn and Boyce, 1963; Miller and Murray, 1962).

Klinefelter syndrome

In spite of the testicular dysgenesis, tumours seem to be very uncommon in the testes of patients with this syndrome. An embryonal cell carcinoma was reported by Beattie (1957). An

interstitial cell tumour was reported by Arduino and Glucksman (1963). The nuclear sex of patients with testicular tumours was investigated by Ashley and Theiss (1958) and found to be male. In an interesting case presented by Klotz et al. (1959), the father, grandfather and grand uncle of a patient with Klinefelter's syndrome had had cancer of the testis. The authors considered, in view of the rarity of malignant tumours in the testes of patients with Klinefelter's syndrome that this strong family history did not justify a prophylactic orchidectomy.

Gonadal dysgenesis

Gonadal dysgenesis, where both gonads are represented by remnants of tissue that do not contain any germ cells, should in a discussion of gonadal tumours, be clearly differentiated from mixed or atypical gonadal dysgenesis, and from the condition of rudimentary testes. In mixed gonadal dysgenesis a testis is present on one side and a primitive undifferentiated gonad or no gonad is on the other side (Sohval, 1964). The replacement of the testis by a tumour, in a patient with mixed gonadal dysgenesis may make its differentiation from gonadal dysgenesis almost impossible. In the syndrome of rudimentary testes, the gonads are rudimentary but contain recognizable testicular tissue (Boczkowski and Teter, 1966).

Tumours have been described in the streak gonads of patients with gonadal dysgenesis but these were all benign and possibly more hyperplastic than neoplastic. In 4 cases described by Stange (1957) the tumours were two cases of hilus cell hyperplasia, an early Brenner tumour and a rete adenoma. Another hilus cell

adenoma in a streak gonad was reported by Warren et al. (1964).

Cases of mixed gonadal dysgenesis with a tumour in the testis have been reported. In these, histological evidence of testicular tissue was still available in spite of the presence of the tumour (Teter and Tarlowski, 1960; Ober, 1962; Reddy and Vittal, 1963; Lewis et al. 1963a, Miller et al. 1960; Naidu et al. 1965; Pinkerton, 1965). In the case of Ober (1962), the ovarian-like stroma in the rudimentary gonad was taken as an evidence for its being an ovary (in the absence of ovarian follicular structures or germ cells), and the intersex state was therefore interpreted as true hermaphroditism. In view of the absence of ovarian parenchymatous tissue, the case is here regarded as one of mixed gonadal dysgenesis.

Tumours in a testis, with the opposite gonad represented also by a rudimentary testis, have been reported (Robinson et al. 1964; Teter et al. 1964b; Philip and Teter, 1964).

The risk of the development of malignancy in the streak gonads of patients with the classical Turner syndrome does not seem to be significant. Dominguez and Greenblatt (1964) reported a case. The case of Toth and Feher (1962), however, was atypical in that an enlarged clitoris was present.

Extra-gonadal tumours in intersexes

Apart from gonadal tumours, it is interesting to note that extra-gonadal tumours have been occasionally noted in intersexes. In some cases this was apparently a fortuitous association as in the case of carcinoma of the caecum reported in a patient with testicular feminization by Tranquade et al. (1962), and that of the bladder

carcinoma in a male pseudohermaphrodite reported by Hammar and Forbes (1965) and that of the extra-gonadal teratomas in Klinefelter's syndrome mentioned by Taylor (1965). Carcinoma in the female genital tract in male pseudohermaphrodites has also been reported (Burkart et al. 1963, Smith, 1964). The incidence of carcinoma of the breast in patients with the klinefelter syndrome was stated to be similar to that in females (Jackson et al. 1965). Mammary carcinoma in a true hermaphrodite was reported by Moriarty (1944). Bilateral fibrocystic disease of the breast with intra-ductal papilloma and epithelial hyperplasia has been reported in a patient with testicular feminization, whose sister, who also never menstruated, died of breast cancer (Caffrey and Fritzlen, 1965). The developmental proximity of the genital ridge and the nephrogenic anlage imparts some interest to the case of Wilms tumour reported in a male pseudohermaphrodite by Stump and Garrett (1954) and to the case of nephroblastoma in association with agonadism reported by Angström (1965). The possible role of hypogonadism in the development of pituitary gland tumours has also been discussed (Kelly, 1963). Willemse (1962) reported a patient as suffering from Turner's syndrome and acromegaly. An interesting point in this latter case was the apparent non-responsiveness of the patient to the excessive amounts of circulating growth hormone. Finally, it may be mentioned that leukemia was noticed to occur much more frequently among individuals with abnormal sex chromosome complements (Lewis et al. 1963b) and their relatives (Miller et al. 1961; Baikie et al. 1961).

2. DISGERMINOMAS AND INTERSEX STATES

Disgerminomas are most commonly found in the ovaries of phenotypically normal women. Pregnancies have both preceded and followed the removal of a disgerminoma (Santesson, 1947), and at least 23 cases of disgerminoma occurring during pregnancy have been reported (Pece, 1964). Figure 127 shows a corpus luteum immediately adjacent to tumour cells. The stromal cells in our five cases were consistently chromatin positive. In our cases the tumour cells were unsuitable for demonstrating sex chromatin, but other authors have found a high incidence of sex chromatin in some disgerminomas (Sohval and Gaines, 1955; Theiss et al. 1960; Hienz, 1961). Finally, we report here the results of chromosome analysis in three patients with a disgerminoma in each of whom there was a normal female karyotype, 46/XX. The evidence appears to be incontrovertible that disgerminomas usually occur in the ovaries of genetic females.

However, it is commonly believed that there is a significant association between ovarian disgerminomas and intersex states and statements to this effect appear in most of the standard textbooks. The series of the 27 disgerminomas in intersex cases collected by Meyer (1925, 1931b) forms the basis for this belief. However, it has not been sufficiently recognized that Meyer used the term disgerminoma to refer to tumours of both the testis and ovary and that his series included no definite cases in female pseudohermaphrodites. In fact, his series includes no example where the gonad which was the site of the tumour could be definitely identified as an ovary, and in only one of his cases was the other gonad shown to be an

ovary. Furthermore, this latter case which was originally reported by Moots (1921) was subsequently considered to be an arrhenoblastoma rather than a disgerminoma (Javert and Finn, 1951). In another four of his cases, Meyer considered that the contralateral gonad was probably an ovary, but this opinion was not based on histological evidence.

This illustrates a peculiar difficulty in analysing the cases reported in the literature. The nature of the gonad bearing the tumour is often obscured by the tumour, and the structure of the contralateral gonad is rarely described. For example, Meyer could identify gonadal sex in only 7 out of his 27 cases.

The application of nuclear sexing and chromosome analysis to this problem might be expected to supplement gonadal sexing based on histological evidence. We have, therefore, collected from the literature 36 cases of tumours of the seminoma-disgerminoma type in intersex patients in which one or more of the following pieces of information was available - a histological report on one or both gonads, sex chromatin analysis, or chromosome analysis of the patient. Fourteen of these tumours were described as seminomas and the other 22 as disgerminomas. Cases reported as embryonal carcinoma, teratocarcinoma and gonadoblastoma have been excluded. A summary of these cases is presented in Table XVI. This provides information on four specific points.

1. Nature of the gonad which contained the tumour:

This was reported as follows:

Testis	15 cases
Ovotestis	3 cases
? Ovary	1 case
Not identified	17 cases

The one case where the site of the tumour was identified as an ovary was described by Long et al. (1941). This diagnosis was apparently based on the finding of ovarian stroma. However, this cortical type of tissue is not specific to the ovary as it can also be found in ectopic testes (Morris and Mahesh, 1963). Two other features of this case suggest a testicular origin for this tumour. The findings of a deep voice, hirsutism and penile erections suggest that the patient was a male, unless it is assumed that the tumour was actively androgenic. Secondly, calcified concretions were present in the tumour. These are practically unknown in ovarian disgerminomas but are common in the tumours of dysgenetic testes (Teter et al., 1964b).

The three cases in which the tumour was found in an ovotestis need further comment. In the case described by Essenberg and Feinberg (1937) the tumour was situated in the testicular part of an ovotestis. In the case originally described by Polano (1921) and reviewed by Meyer (1925, 1931b), one gonad was replaced by the tumour, while in the contralateral gonad, an ovotestis, Meyer found a focus of disgerminoma cells in the ovarian component. Meyer considered that this was a separate primary disgerminoma arising in ovarian tissue, but it is difficult to exclude the possibility that it may have been a metastatic deposit from the other gonad. In the case of Botella-Llusia (1960) an early focus of what were apparently disgerminoma cells was found between the testicular and ovarian components of one ovotestis. In this case, the origin of the tumour cannot be definitely ascribed to either testis or ovary.

One can conclude from these data that no case of intersex with a disgerminoma has been reported where the tumour has been

proven to have arisen in ovarian tissue. Undoubtedly, the vast majority have arisen in testicular tissue.

2. Nature of the other gonad:

The nature of the gonad on the side opposite to the tumour was reported as follows:

Testis	12 cases
Ovotestis	4 cases
Ovary	2 cases
Not identified	18 cases

The two cases in which the other gonad was described as an ovary were those of Essenberg and Feinberg (1937) and Stirling (1959). In both cases the patient was a true hermaphrodite and the tumour developed in a testis.

Two of the cases in which the nature of the gonad was not identified deserve comment. In the case of Corriden (1949) both gonads were replaced by tumour tissue. The gonad contained a disgerminoma, and the author describes an epididymis attached to this tumour. The other gonad was replaced by a typically ovarian type of tumour, a mucinous cystadenoma, and this suggests that this gonad was an ovary. This is most probably an example of true hermaphroditism (Melicow and Uson, 1959). Moehlig's case (1942) was unusual. One gonad, a testis, contained a disgerminoma, and there were two other separate gonads in the pelvis. One of these was removed and found to be a hypoplastic testis. The other gonad was thought to be an ovary, but it was neither removed nor was a biopsy taken.

One can conclude that in many intersex cases with a disgerminoma, the contra-lateral gonad is a testis. In cases, in

which the contralateral gonad proved to be either a normal ovary or an ovotestis, it is likely that the gonad which was the site of the tumour was either wholly or partly testicular - i.e. all of these patients were probably true hermaphrodites.

3. Sex chromatin studies:

There is information about sex chromatin in 19 patients of whom 16 were chromatin negative and three were chromatin positive.

The three chromatin positive cases require further comment. In one case (Philipp and Stange, 1960), only 23 per cent of nuclei from a buccal smear were chromatin positive. This low count is suggestive of some form of chromosomal mosaicism rather than a simple XX sex chromosome complement. Furthermore, the contralateral gonad in this case was rudimentary. In the second case (Teter and Tarlowski, 1960) the tumour replaced one gonad while the contralateral gonad was again rudimentary. The presence of calcification in this tumour suggests that it was testicular in origin. In neither of these two cases was chromosome analysis performed. The third case (Botella-Llusia, 1960) was a true hermaphrodite with bilateral ovotestis. This patient was found to have a normal female karyotype, 46/XX.

One can conclude that the majority of intersex patients with a disgerminoma have been chromatin negative. The three chromatin positive patients which have been reported were probably either true hermaphrodites with testicular tissue or chromosome mosaics in association with dysgenetic gonads. One feature common to all of these patients is likely to have been the presence of testicular tissue.

4. Chromosome analysis:

Chromosome analysis was carried out in 9 cases and the following sex chromosome complements were found:

XY	2 cases
XO/XY	4 cases
XO	1 case
XX/XY	1 case
XX	1 case

The one case with an apparently normal female karyotype has already been discussed (Botella-Llusia, 1960), and shown to be an example of true hermaphrodite with a 46/XX karyotype. The presence of testicular tissue despite the apparent absence of a Y chromosome has already been well documented (Harnden and Armstrong, 1959). With this exception, no intersex state with a disgerminoma and a normal female karyotype has yet been described. Furthermore, every case has had an XY cell line, with the further exception of one patient with a 45/XO karyotype.

These four observations lead to the conclusion that in patients with an intersex state there has been no clearly documented example in which a disgerminoma has originated in ovarian tissue. Undoubtedly, in the majority of cases it was shown to derive from testicular tissue. As a corollary to this statement, there is no well established case of a female pseudohermaphrodite with a disgerminoma, the patients reported being either male pseudohermaphrodites or true hermaphrodites. The liability of a female pseudohermaphrodite or a true hermaphrodite to develop an ovarian disgerminoma is probably the same as in women with normal sex development.

However, tumours other than disgerminoma have occasionally

been found in the ovaries of intersex patients. Examples of mucinous cystadenomas (Corriden, 1949; Bani, 1956; Gresham and Fairgrieve, 1960), Brenner tumour (Weed et al., 1947; Stange, 1957), thecoma (Vaughn and Gonzales-Angulo, 1961), granulosa-theca cell tumour (Hauser et al., 1960), and dermoid cyst (Claret and McIntosh, 1954), have been reported. The small number of such case reports, however, suggests that the risks of any type of neoplastic transformation in the ovary may not be increased by the existence of an intersex state.

On the other hand, the incidence of testicular tumours is undoubtedly increased in patients with an intersex state. Cryptorchidism is common in these cases, and the liability of ectopic testes to neoplasia is well documented (Dixon and Moore, 1953). It is significant that in these cases the tumour, although commonly a seminoma, is not infrequently a teratoma, embryonal carcinoma, teratocarcinoma or chorionepithelioma, as we have mentioned in part I of this discussion. In one series of 61 testicular tumours in male pseudohermaphrodites, there were 38 seminomas, 14 teratomas, and 9 miscellaneous tumours (Gilbert, 1942).

The remarkably close association between the gonadoblastoma and intersex states is now well documented. Of the cases described, so far, all were associated with abnormal sex development, all proved to be chromatin negative, and in all the cases in which chromosome analysis is available an XY cell line was reported (Philip and Teter, 1964). It seems likely that the gonadoblastoma is a variant of the testicular seminoma rather than the ovarian dysgerminoma, being a tumour of dysgenetic testes.

The age distribution of seminomas and disgerminomas is relevant to this argument. Testicular seminomas are relatively rare in younger age groups, only 12 per cent being found in males under the age of 30 (Fergusson, 1962). By contrast, 80 per cent ovarian disgerminomas, in patients with normal sex development, occur before the age of 30 years (Santesson, 1947). However, many of the disgerminomas which have been reported in intersex cases were found in older age groups, and the age distribution in these is more comparable to that of testicular seminomas rather than ovarian disgerminomas (Willis, 1960). This may support the belief that these tumours in intersex cases are closely related to the seminoma type of tumour.

Apart from the association with intersex states, few cases have been reported as ovarian disgerminoma in association with masculinization (Gough, 1938; Seegar, 1938; Ber, 1949; Plate, 1953; Usizima, 1956). Careful analysis of these cases, however, shows that they were probably intersexes with the virilization due not to the tumour itself but to a testicular gonad in which the tumour developed. The removal of the gonad and its tumour naturally resulted in amelioration of symptoms. Menstruation was not stated to be established in any single case, although the youngest patient was 15.5 years old. The presence of ovarian tissue was also not established by histological examination in any of the cases. The opposite gonad was described as rudimentary in two of the cases (Gough, 1938; Plate, 1953). In the case of Plate (1953), subsequent chromosomal analysis showed that the patient had a 46/XY pattern (Scott, 1965).

A simple concept of the relation of the disgerminoma-seminoma

type of tumour to intersex states emerges from this review. These tumours undoubtedly occur most commonly in the testes or ovaries of patients with normal sex development. However, the risk of developing a seminoma is increased in ectopic testes whether or not there is an associated intersex state. The incidence of other forms of neoplasms is also increased in ectopic testes, but probably to a lesser extent (Fergusson, 1962). This implies that the significant aetiological factor is possibly the site of the testis rather than the abnormal sex development. Furthermore, there is no evidence that the incidence of neoplasia is increased in the ovaries of intersex patients.

This argument has a clinical application. In intersex states there is an increased risk of gonadal neoplasia only if testicular tissue is present. This may be indicated by the presence of an XY cell line which is strong presumptive evidence of testicular tissue. However, it is important to realize that the finding of a normal female karyotype does not exclude the presence of testicular tissue, for, as we have shown, a true hermaphrodite possessing both ovarian and testicular tissue may have an XX sex chromosome complement. As a corollary to this, the finding of a tumour of the seminoma-disgerminoma type in an intersex patient is strong evidence for the presence of testicular tissue.

3. ARRHENOBLASTOMA AND INTERSEX STATES

Pick (1905) reported the first description of an extremely well-differentiated arrhenoblastoma in a female patient. He remarked on its close resemblance to the typical tubular adenoma of the undescended testis, and suggested that his case may in fact have been a testicular tumour arising from an ovotestis. However, Meyer (1931) included Pick's tumour and similar cases as examples of well-differentiated arrhenoblastomas, and the status of the tubular adenoma of Pick as an ovarian tumour has been generally accepted. However, with the recognition of the testicular feminization syndrome (Morris, 1953), it is now appreciated that the presence of bilateral cryptorchidism is compatible with a female phenotype. Moreover, in this syndrome, the undescended testes are commonly the site of tubular adenomas which may replace the whole testis. In these cases, if the opposite gonad is not examined, or if it is also replaced by the tumour, an erroneous diagnosis of ovarian arrhenoblastoma may be made. This risk is increased by the common finding of an ovarian-like stroma in the capsule of these testes (Morris and Mahesh, 1963).

It is impossible to know how frequently this error has been made. In two of our cases in which an arrhenoblastoma was initially reported, the correct diagnosis proved to be testicular tubular adenomas in genetic males. In an incomplete review of the literature, we have encountered nine cases reported as ovarian tumours in females where the true diagnosis may be testicular feminization (Behrend and Levine, 1936; Anderson, 1937; Dudman, 1939; Novak, 1943 - 2 cases; Jolles and Gleave, 1945; Goldberg

and Maxwell, 1947; Javert and Finn, 1951; Langley, 1954). The features common to these cases are primary amenorrhoea, absence of masculinization, and an absent or rudimentary uterus.

Sex chromatin analysis will clarify the diagnosis in most of these cases. As we have shown, ovarian arrhenoblastomas are chromatin positive, while testicular tubular adenomas are chromatin negative. It should be emphasized that the histological appearance of a testicular tubular adenoma and a well-differentiated ovarian arrhenoblastoma may be indistinguishable. Not all of this type of tumour occur in male testes, and a number of cases have been described in parous women (Javert and Finn, 1951). This emphasises the value of routine sex chromatin analysis as an adjunct to histological examination in elucidating the nature of these tumours.

SUMMARY

1. A study of five cases of ovarian disgerminoma and five cases of arrhenoblastoma is presented.
2. All five patients with a disgerminoma were phenotypically normal females and had a normal female sex chromatin pattern, while three of them had a normal female karyotype. Of the five tumours originally reported as arrhenoblastomas, two proved to be testicular tubular adenoma replacing the undescended testes of genetic males with the testicular feminization syndrome.
3. A review is presented of 36 reported cases of intersex with tumours of the seminoma-disgerminoma type with special reference to the nature of the ipsilateral and contralateral gonads, and the sex chromatin and chromosome analysis of the patient. It is concluded that no disgerminoma occurring in an intersex patient has been proven to have arisen in ovarian tissue, while in the majority of cases it has been shown to have originated in testicular tissue.
4. There is probably no increased risk of any type of neoplastic change in the ovaries of intersex patients. By contrast, there is an increased risk of testicular tumours, including the seminoma, in male intersexes. This is thought to be a function of the ectopic position of the testes rather than the abnormal sex development per se.
5. The histological similarity of the well-differentiated arrhenoblastoma and the testicular tubular adenoma may lead to errors in diagnosis. Sex chromatin analysis is invaluable in these cases.

TABLE XVI

Tumours of the Disgerminoma-Seminoma Type in Intersexes

Author	Tumour nomenclature	Gonadal sex		Genetic sex	
		Tumour side	Contra-lateral	Sex chromatin	chromosome analysis
Unger (1905)	Disgerminoma ¹	Testis	Testis		
Marion (1905)	Disgerminoma ¹		Testis		
Zacharias (1909)	Disgerminoma ¹		Testis		
Skajaa (1919)	Disgerminoma ¹		Testis		
Polano (1921)	Disgerminoma ¹	Ovotestis ²	Ovotestis		
Menetrier (case 1) (1922)	Seminoma	Testis	Testis		
Reverdin (1923)	Disgerminoma ³		Ovotestis		
Weyeneth (1936)	Disgerminoma		Ovotestis		
Essenberg and Feinberg (1937)	Seminoma (Carcinoma)	Ovotestis	Ovary		
Seegar (1938)	Disgerminoma	Testis	Testis		
Long et al. (1941)	Disgerminoma	? Ovary ²			
Moehlig (1942)	Seminoma	Testis	Testis ² 2		
Corriden (1949)	Disgerminoma		Testis		
Lafferty and Pendergrass (1950)	Seminoma	Testis			
Huddleston-Slater (1953)	Disgerminoma			Negative	
Morris (1953)	Seminoma (Disgerminoma)	Testis			
Plate (1953)	Disgerminoma			Negative ⁴	46/XY ⁵
Spielman and Motylloff (1955)	Disgerminoma		Testis		

TABLE XVI (Contd.)

Author	Tumour nomenclature	Gonadal sex		Genetic sex	
		Tumour side	Contra-lateral	Sex chromatin	Chromosome analysis
Melicow and Uson (case 3) (1959) (case 4) (1959)	Seminoma Disgerminoma Seminoma	Testis Testis	Testis Ovary	Negative Negative Negative	
Teter and Tarlowski (case 1) (1960) (case 2) (1960)	Disgerminoma (Seminoma) Disgerminoma ⁶ Disgerminoma ⁶			Negative Positive Positive	46/XX XO/XY XO/XY
Botella-Llusia (1960) Miller et al. (1960)	Seminoma Seminoma	Ovotestis Testis Testis	Ovotestis	Negative Negative Positive	45/XO ⁷ XO/XY or XO/XY/XX
Philipp and Stange (1960) Dominguez and Greenblatt (1962) Goodlin (1962)	Disgerminoma Disgerminoma Seminoma	Testis Testis Testis		Negative Negative Negative	
Reddy and Vittal (case 3) (1963) Hauser (1963) Halley (1963) Lewis et al. (1963) Teter et al. (1964) Sohval (1964) Bain and Scott (1965)	Seminoma Seminoma Seminoma Seminoma Disgerminoma Disgerminoma Disgerminoma	Testis Testis Testis Testis Testis Testis Testis	Testis Testis	Negative Negative Negative Negative Negative Negative Negative	XO/XY 46/XY XX/XY

1. Nomenclature after Meyer (1925)
2. Details in text
3. Nomenclature after Neumann (1927)
4. Sex chromatin after Carpentier (1956a)
5. Chromosome analysis after Scott (1965)
6. Tumour nomenclature after Overzier (1963)
7. Chromosome analysis after Greenblatt et al. (1967).

SUMMARY

1. A consecutive series of 1734 ovarian tumours was studied.
2. Data about the incidence, age distribution, bilaterality and the relative frequency of the various tumour types were presented. The pathological diagnosis was re-assessed and the tumours were re-classified. Observations on individual types of ovarian tumours were presented.
3. The possible origin of malignant tumours in ovarian endometriosis was investigated in our series of 418 primary ovarian tumours and in a series of 592 cases of ovarian endometriosis examined during the same period. The literature was also reviewed in order to elucidate the clinical and pathological features of these cases.
4. Attention was directed to the frequency with which granulosa and more particularly theca cell tumours may be present for long periods without causing clinical enlargement of the ovary. A clinico-pathological study of a series of 25 such cases was presented.
5. Certain very rare types of ovarian tumours were diagnosed during the re-evaluation of the diagnosis in our cases. The following tumour types were presented in detail: primary mesodermal mixed tumours, malignant Brenner tumour, malignant adenofibroma, malignant well differentiated teratoma and mesoblastoma.
6. An interesting case of ovarian enlargement simulating tumour formation in a virilized patient during pregnancy was studied and discussed.

7. Cytogenetic techniques were applied to the study of the problem of the histogenesis of teratomas. Nuclear sex was studied in 187 cases of benign ovarian teratomas and chromosome analysis was performed in 6 cases. The bearing of the results on the problem of the origin of teratomas was discussed.
8. The relationship between ovarian tumours and intersex states, with special reference to the disgerminoma and arrhenoblastoma, was investigated, in the light of the modern cytogenetic advances. The evidence was presented that there was probably no increased risk of any type of neoplastic change in the ovaries of intersex patients. By contrast, there is an increased risk of testicular tumours, including the seminoma, in male intersexes. This is thought to be a function of the ectopic position of the testis rather than the abnormal sex development per se. The histological similarity of the well-differentiated arrhenoblastoma and the testicular tubular adenoma may lead to errors in diagnosis. Sex chromatin analysis proved invaluable in these cases.

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ATLAS - OF - ILLUSTRATIONS

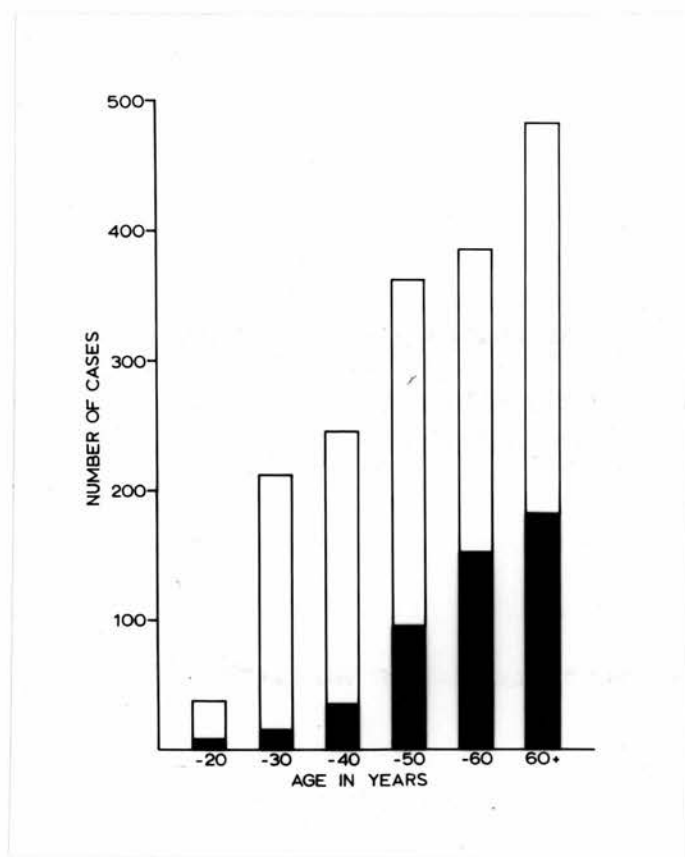


Fig. 1

 Benign
 Malignant

Fig. 2 : Percentage of malignancy in ovarian tumours in the various age groups.

The following may be noted :

1. At no age period was the chance of an ovarian tumour to be malignant more than or equal to its chance of being benign.
2. The percentage of malignancy was quite high below the age of 20 years and after the age of 40 years.

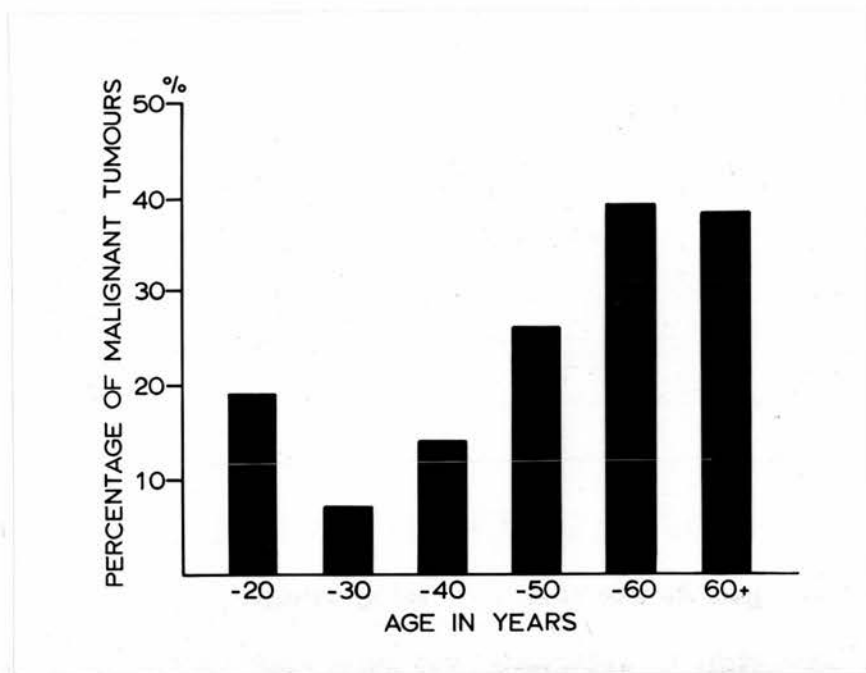


Fig. 2

Fig. 3 : Bilateral ovarian tumours, benign and malignant in the various age groups.

Note : A bilateral ovarian tumour was more commonly malignant in the fourth and fifth decades only. Earlier, the bilateral tumours were more commonly benign. Later, the chance was almost equal.

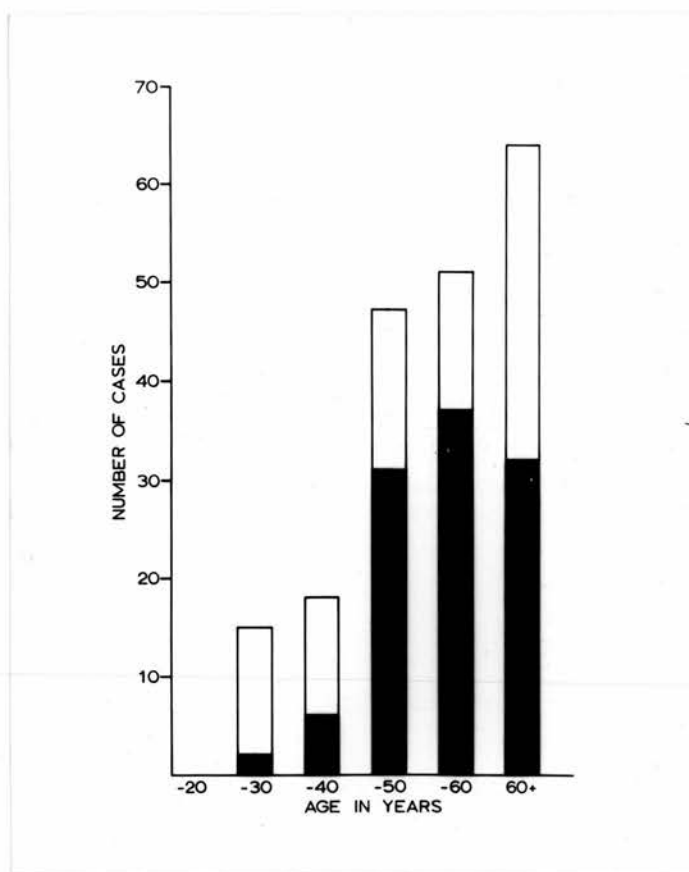


Fig. 3

 Benign
 Malignant

HISTOLOGIC CLASSIFICATION OF THE PRIMARY COMMON EPITHELIAL TUMOURS

1. Serous cystomas

Fig. 4 : Serous benign cystadenoma. X 25

A psammoma body is also seen.

Fig. 5 : Serous cystadenoma with proliferative activity of the epithelial cells (and nuclear abnormalities) but with no infiltrative destructive growth. X 40.

Low potential malignancy.

Fig. 6: Serous cystadenocarcinoma. X 40.

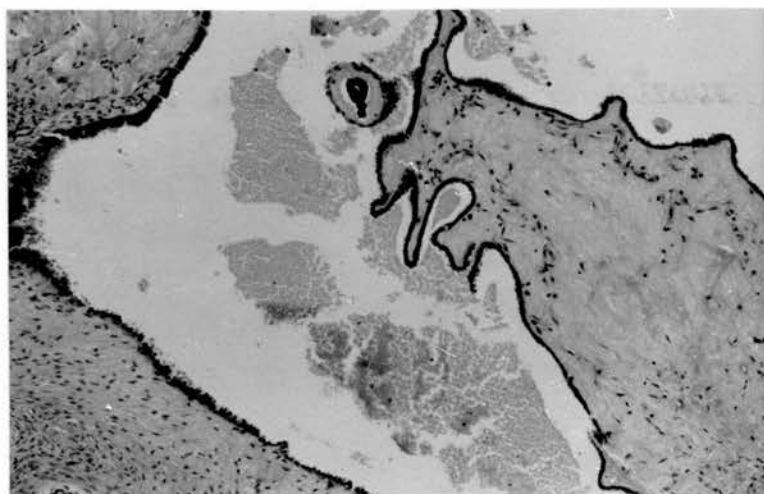


Fig. 4

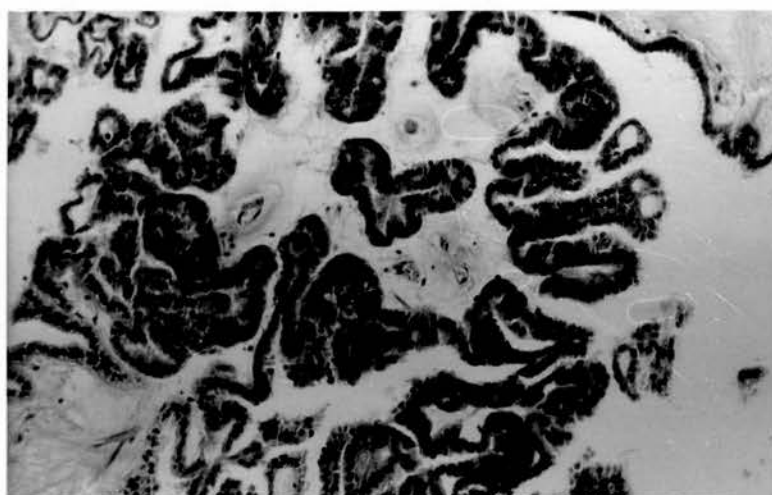


Fig. 5

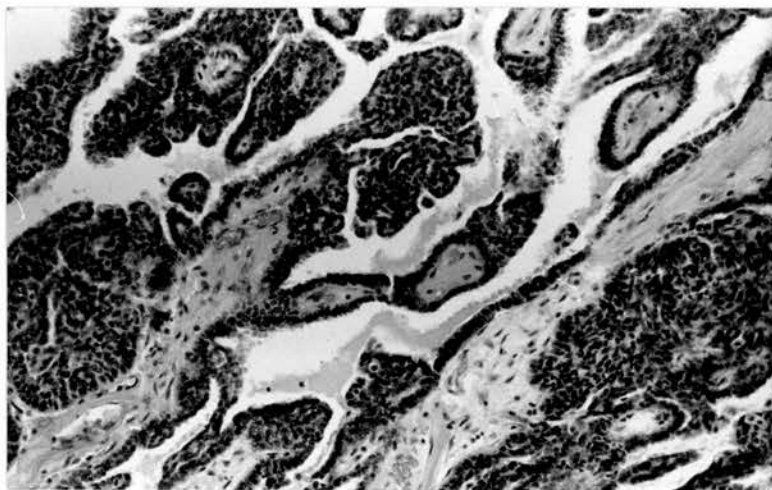


Fig. 6

2. Mucinous cystomas

Fig. 7 : Mucinous benign cystadenoma. X 40.

Fig. 8 : Mucinous cystadenoma with proliferative activity of the epithelial cells (and nuclear abnormalities) but with no infiltrative destructive growth. X 40.
Low potential malignancy.

Fig. 9 : Mucinous cystadenocarcinoma. X 40

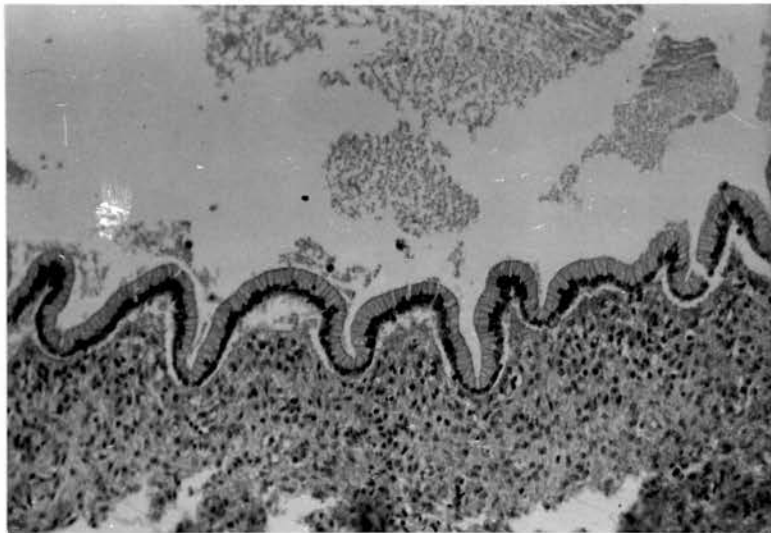


Fig. 7

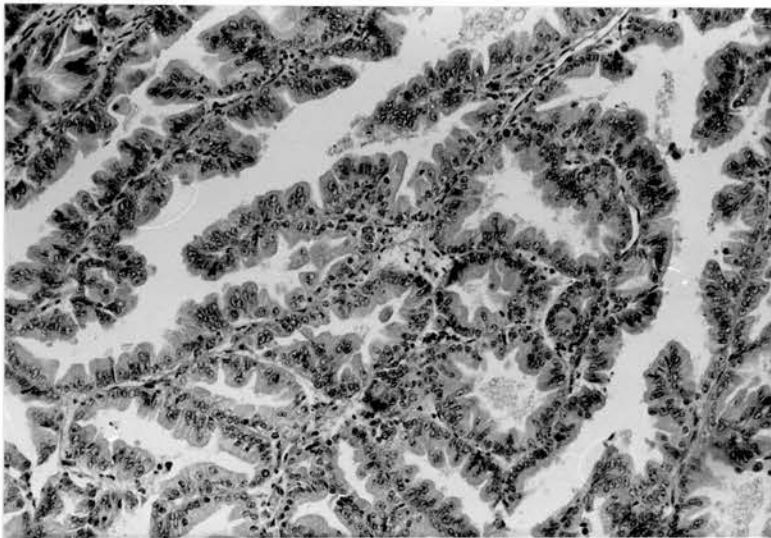


Fig.8

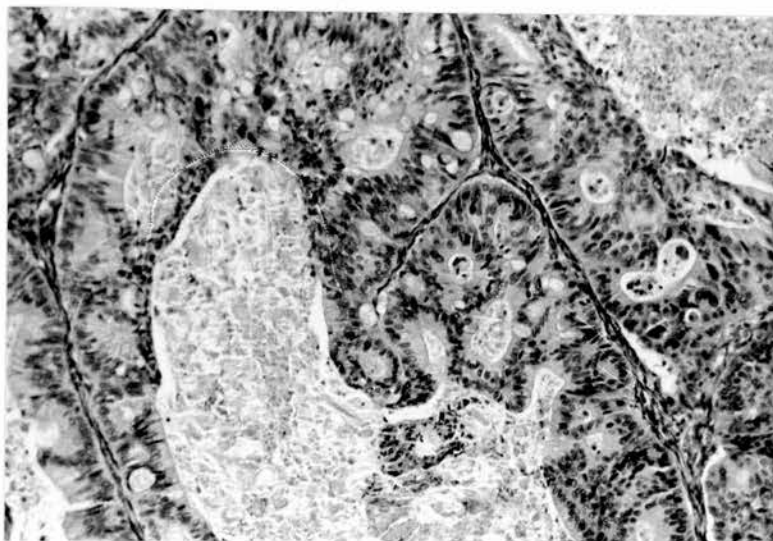


Fig. 9

3. Endometrioid tumours

Fig. 10

Endometrioid tumour with proliferative activity of the epithelial cells but with no infiltrative destructive growth. Low potential malignancy. X 40.

Fig. 11

Endometrioid adenocarcinoma. X 40.

4. Unclassified carcinoma

Fig. 12

Undifferentiated carcinoma (unclassified). X 250.

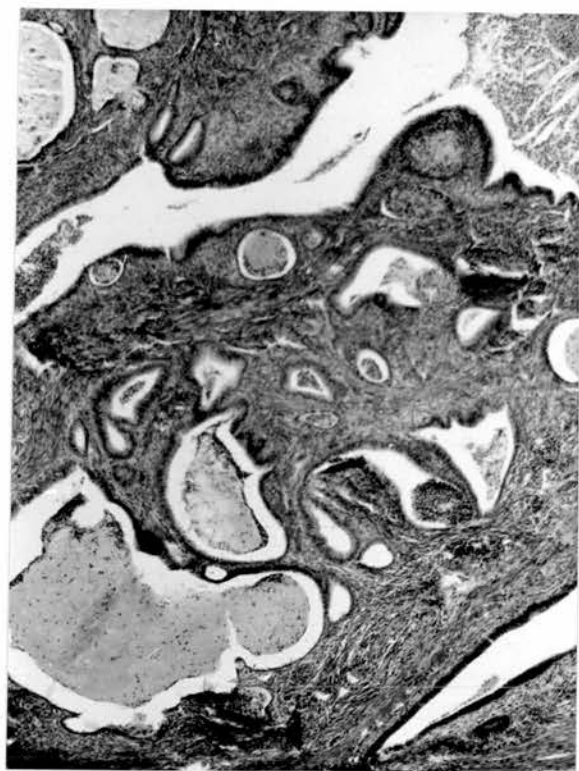


Fig. 10

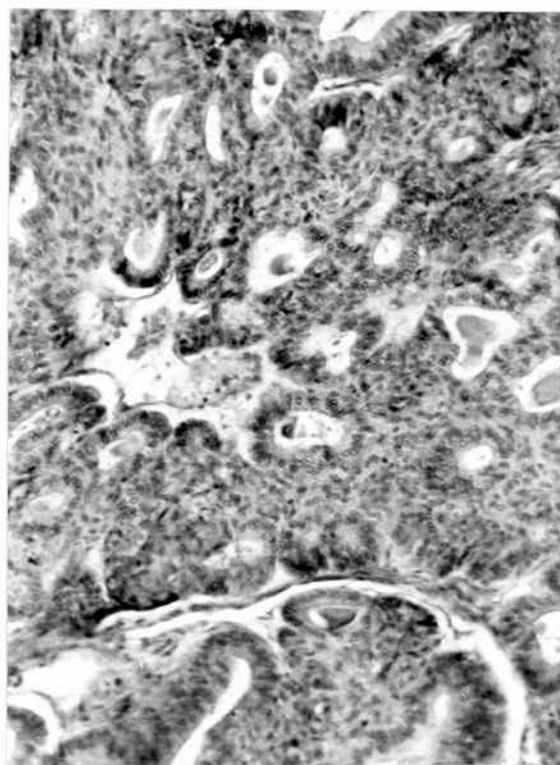


Fig. 11

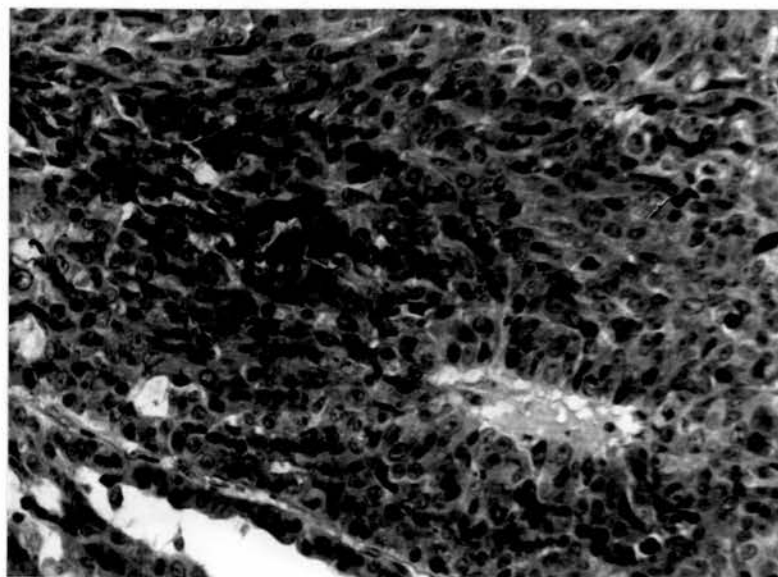


Fig. 12

Epithelial tumours of mixed types.

Fig. 13 : Mucinous (right) and serous (left) areas in a benign cystadenoma. X 40.

Fig. 14 and 15 :

Ovarian carcinoma showing serous (Fig. 14), mucinous (Fig. 15, left)
and endometrioid (Fig. 15, right) areas. X 40.

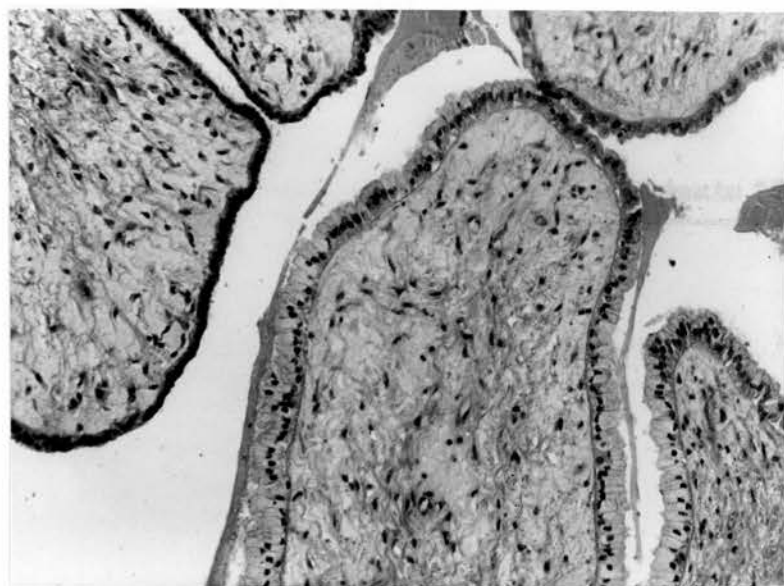


Fig.13

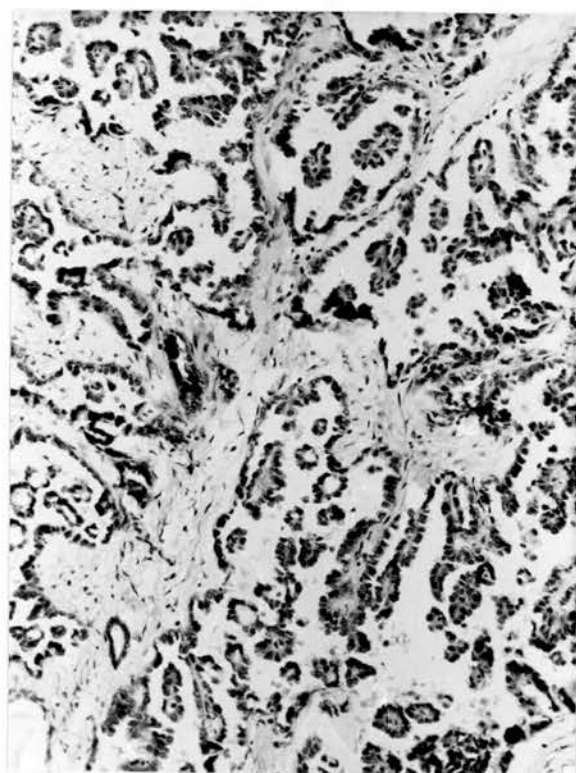


Fig. 14

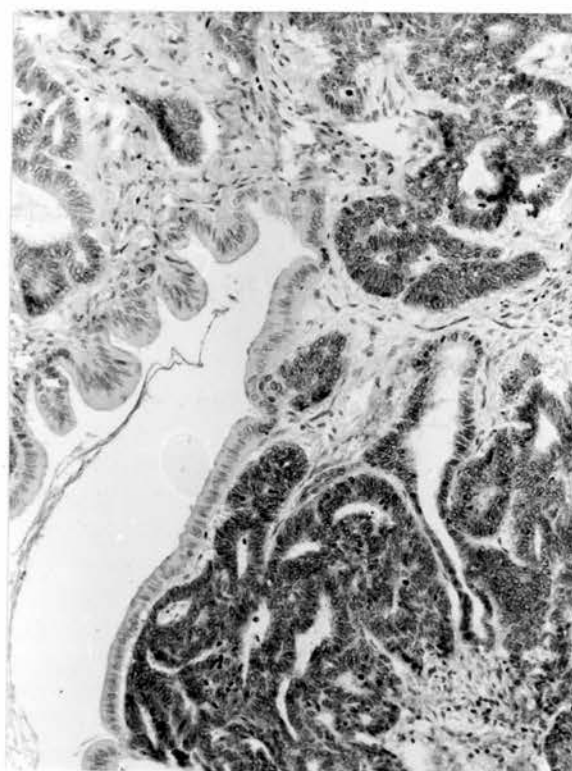


Fig.15

Completely undifferentiated ovarian carcinomas are rare. Examination of other parts of the tumour often shows more differentiated areas. Fig. 16 and 17 illustrate such an extreme example.

Fig. 16 : Areas in an ovarian carcinoma showing complete undifferentiation. X 40.

Fig. 17 : Another area in the same tumour , showing well differentiated adenocarcinoma. X 40.

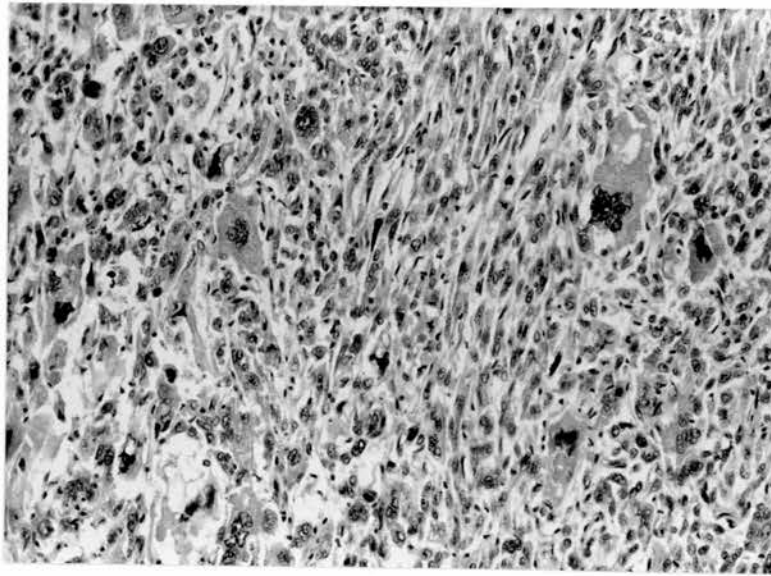


Fig.16

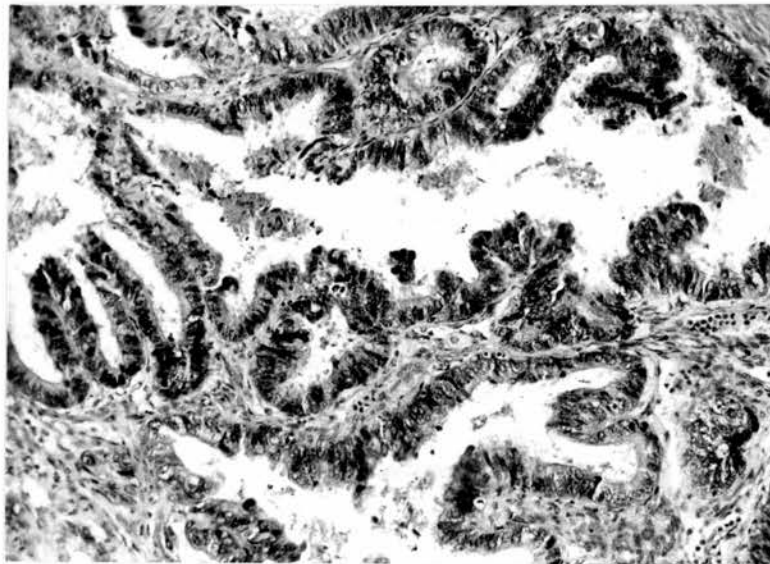


Fig. 17

OVARIAN TERATOMA

The predilection of the benign cystic teratoma to the childbearing period of age is well known. The association with pregnancy is also relatively not infrequent. Fig. 18 illustrates such an example.

Fig. 18 : Benign cystic teratoma removed during pregnancy, showing the presence of decidual reaction beneath the ovarian serosa (lower part of the figure). X 25.

Striking examples of the phenomenon of tissue correlation are often seen in the benign teratoma. In fact, the well known association of sebaceous glands, hair follicles, sweat glands and adipose tissue with stratified squamous epithelium is such an example. Some further interesting examples are shown in the following 3 figures.

Fig. 19 : Respiratory epithelium, cartilage, salivary glands and thyroid tissue in a benign cystic teratoma. X 40.

Fig. 20 : The association of bone marrow with bone in a benign cystic teratoma. X 40.

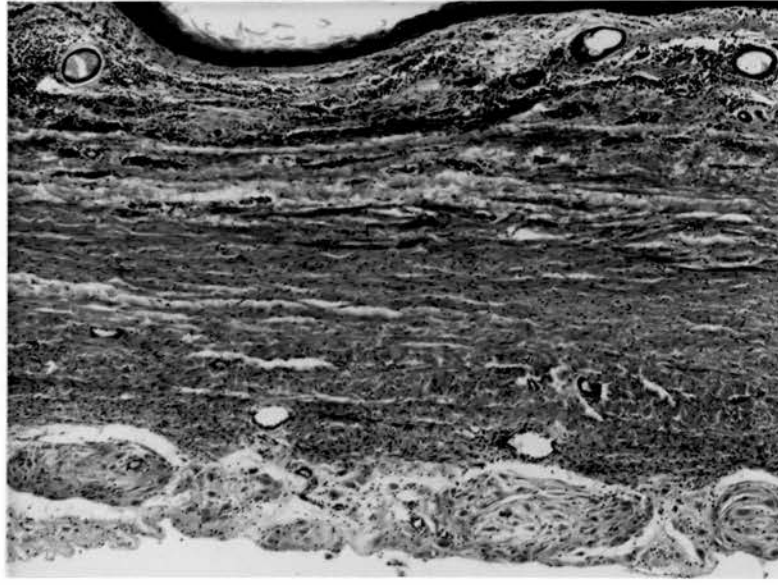


Fig.18

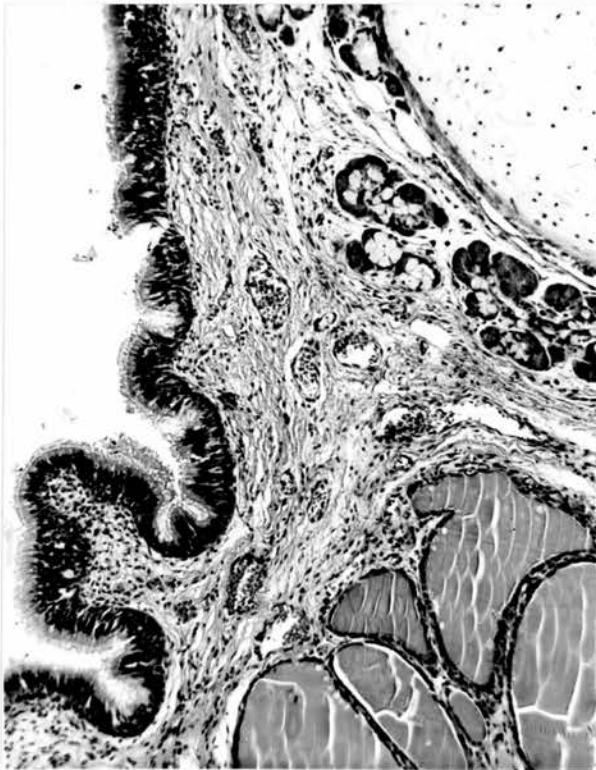


Fig.19

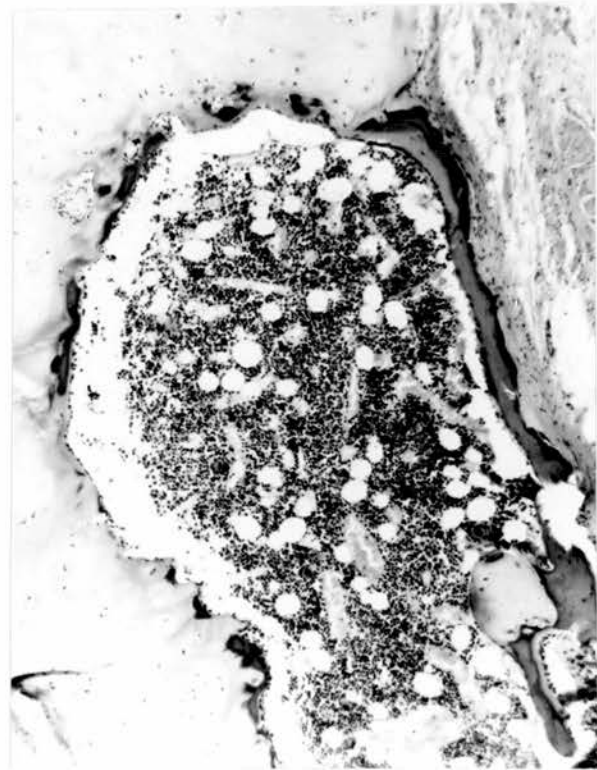


Fig. 20

Fig. 21 : The association of brain tissue and chorioid plexus in a benign cystic teratoma.

X 40.

These examples of tissue correlation may be looked upon as abortive attempts towards organ formation.

Malignant transformation in a benign cystic teratoma indicates that anaplastic changes are confined to a specific tissue element or portion of that element, with the remaining tissues in the tumour continuing to exhibit benign properties. This type should be sharply distinguished from the solid malignant teratoma. Three examples of malignant transformation in a benign cystic teratoma will be illustrated in the following figures : a squamous cell carcinoma, a mucinous carcinoma and an argentaffine carcinoma.

Fig. 22 : Squamous cell carcinoma arising in a benign cystic teratoma. X 40.

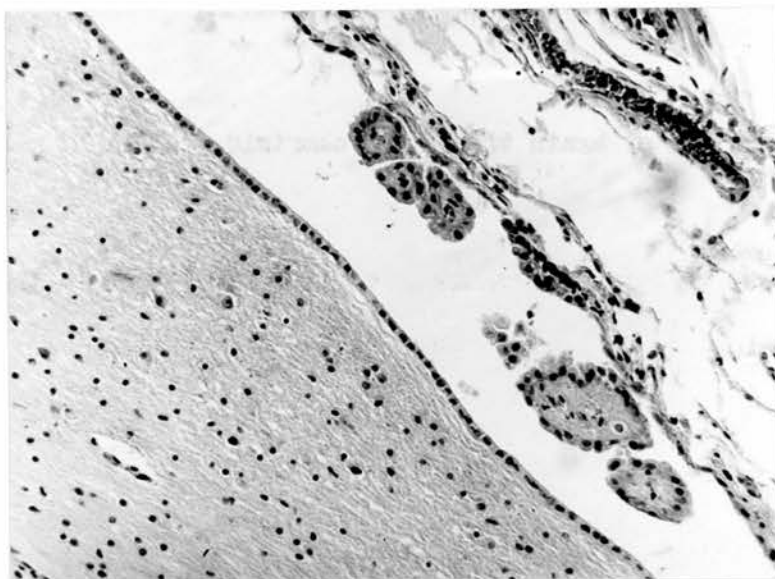


Fig. 21

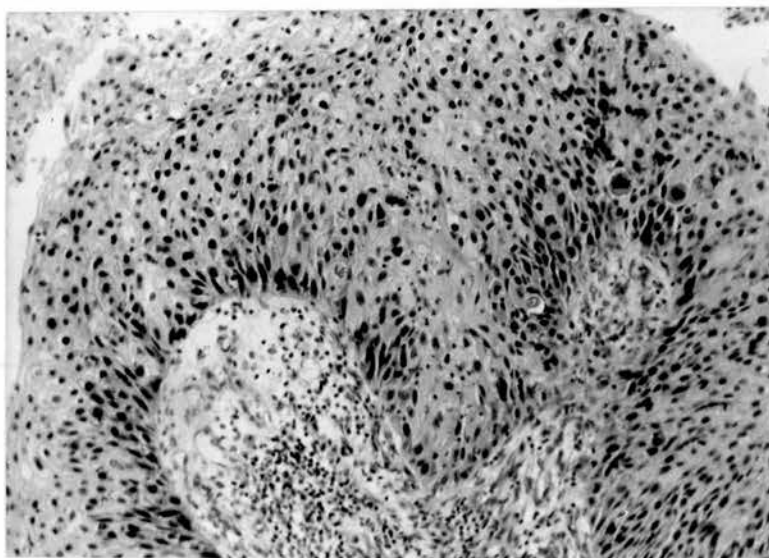


Fig. 22

Fig. 23 and 24 show a mucinous carcinoma arising in a benign cystic teratoma.

Fig. 23 : Active mucinous epithelium lining a gut-like structure (left) in
a benign cystic teratoma. X 25.

Fig. 24 : Mucinous carcinoma , presumably arising from the gut-like structure
shown in the previous figure. X 40.

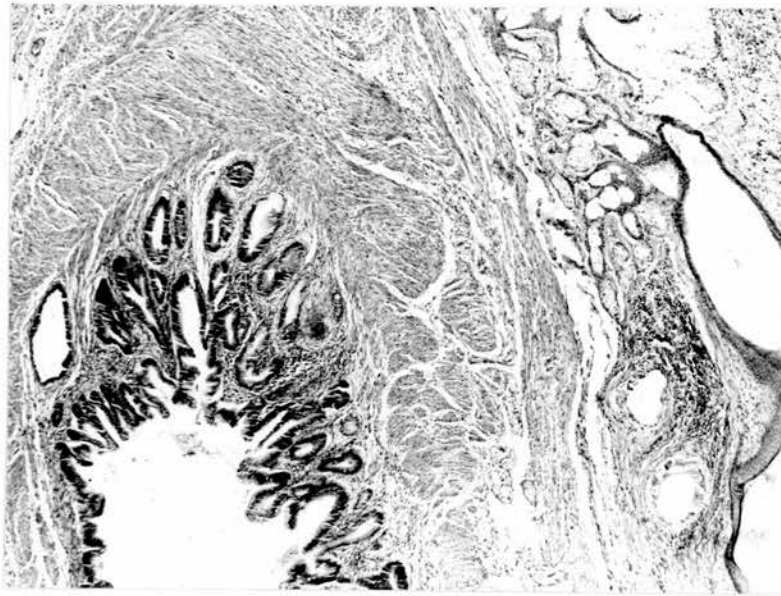


Fig. 23

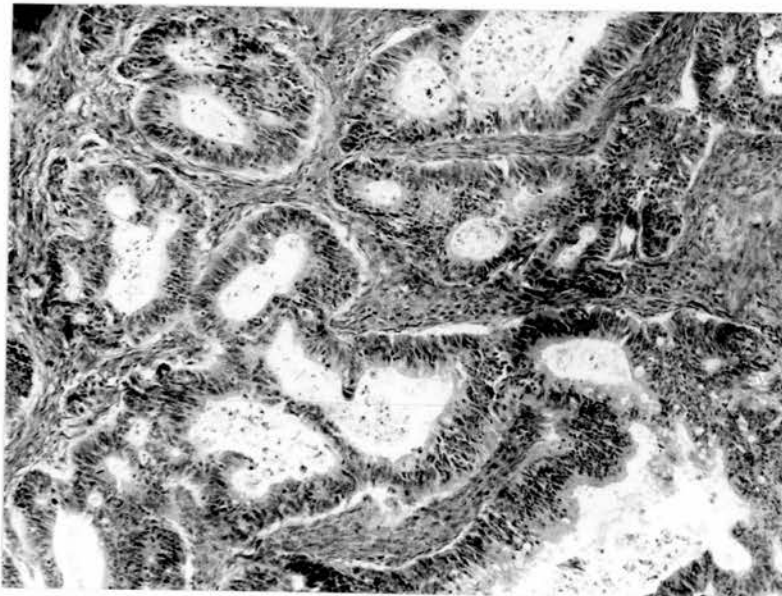


Fig. 24

Argentaffine carcinoma arising in a benign cystic teratoma

Fig. 25 : Gross appearance of the tumour. The solid malignant tumour is shown to the right, as a kidney-shaped mass. The relatively large cyst is the benign cystic teratoma. Hairs can be seen. The dark area (upper left) is an artifact caused by sebaceous material.

Fig. 26 : Argentaffine carcinoma (carcinoid tumour) is seen below in relation to the stratified squamous epithelium of the teratoma (above). X 20

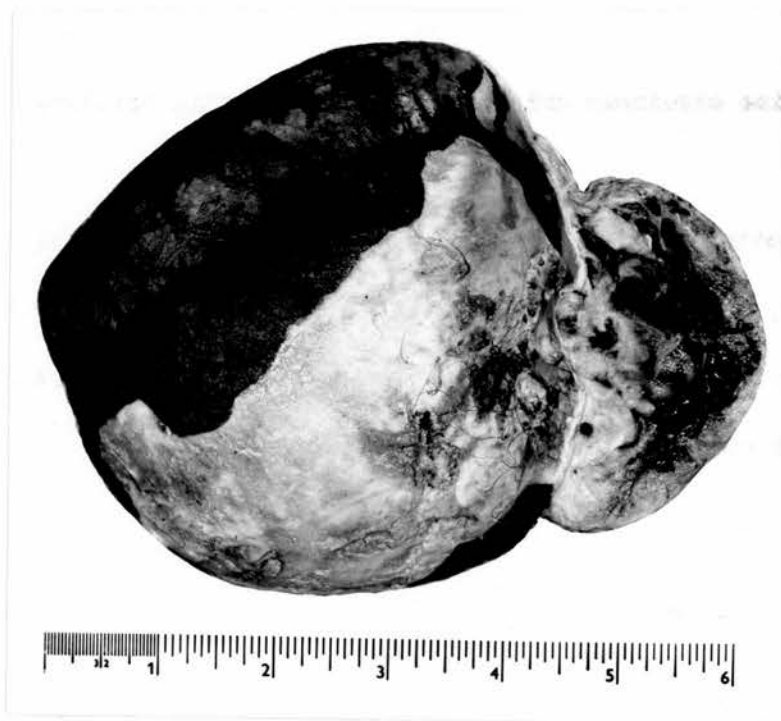


Fig. 25

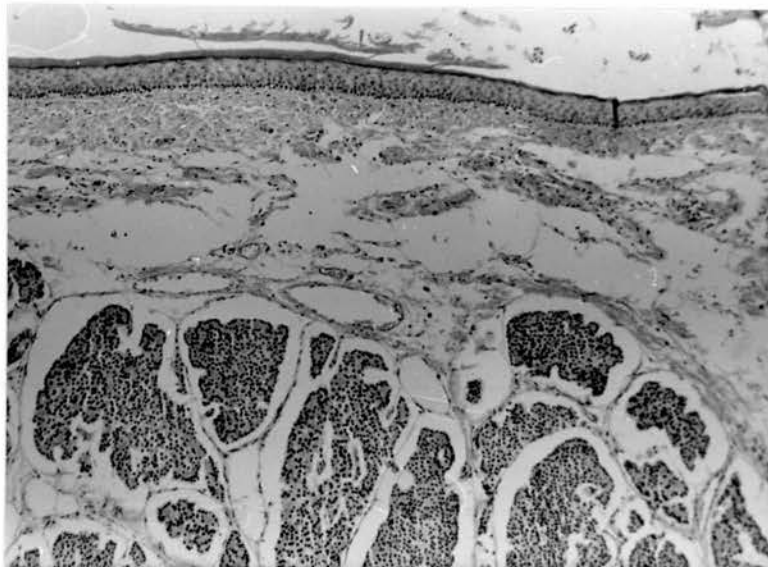


Fig. 26

Solid malignant teratoma of the ovary

Solid malignant teratomas of the ovary are tumours composed of a mixture of tissue forms, both mesenchymal and epithelial, of a variable degree of maturity. Two examples are illustrated here : In one, most of the tissues of the tumour were poorly differentiated. In the other, the teratomatous elements were more or less mature and well-differentiated.

Fig. 27, 28 and 29 are from a solid malignant teratoma, in a girl aged 10 years.

Fig. 27 : Mature epidermal tissue in the teratoma. X 63.

Fig. 28 : Anaplastic areas in the teratoma, with a superficial resemblance to trophoblast. X 63.

Fig. 29 : Another area in the teratoma with superficial resemblance to disgerminoma. X 63.

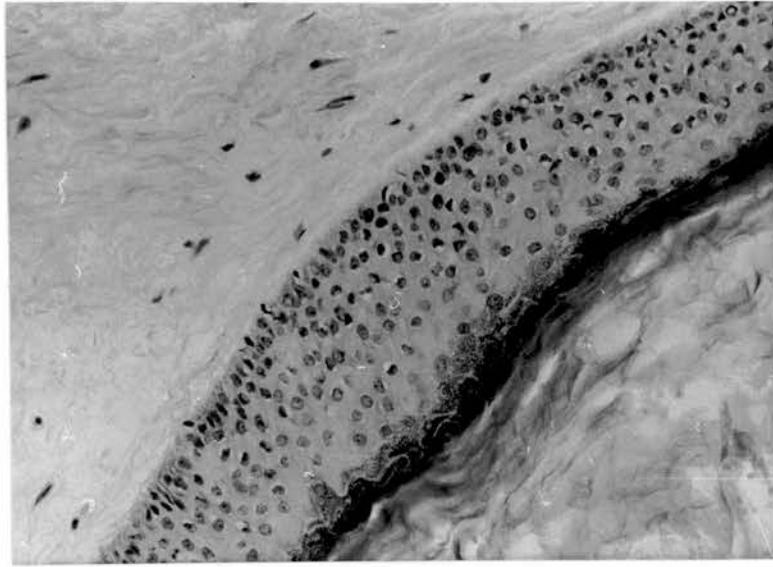


Fig. 27

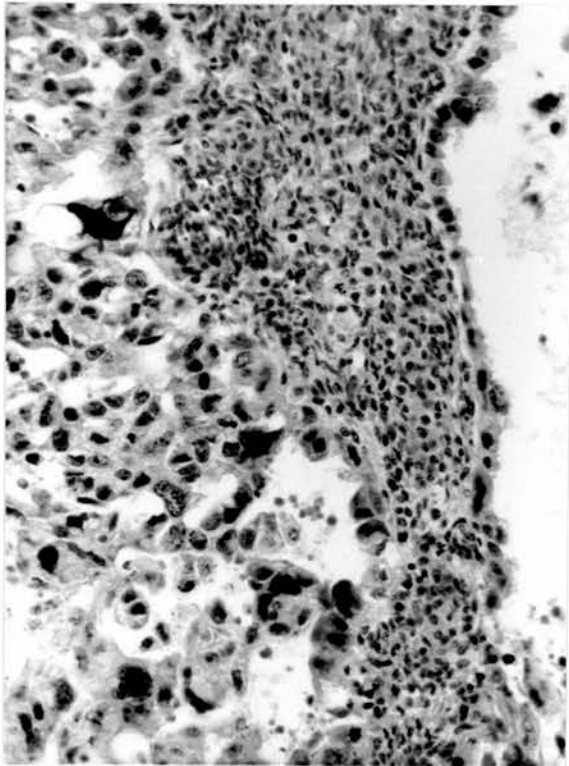


Fig. 28

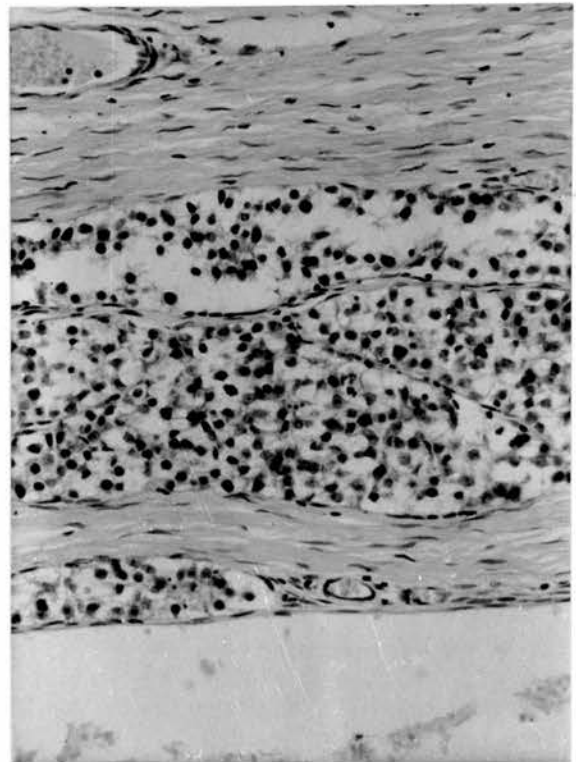


Fig. 29

Figures 30 - 34 are from a solid teratoma removed from a patient aged 39 years.

Fig. 30 : Gross appearance of the tumour. The tumour is partly solid and partly cystic.

Fig. 31 : Neuroglial tissue lining the cystic part of the teratoma (not stratified squamous epithelium as is usual in benign cystic teratomas). X 40.

Fig. 32 : Mature skin elements in the teratoma. X 40.

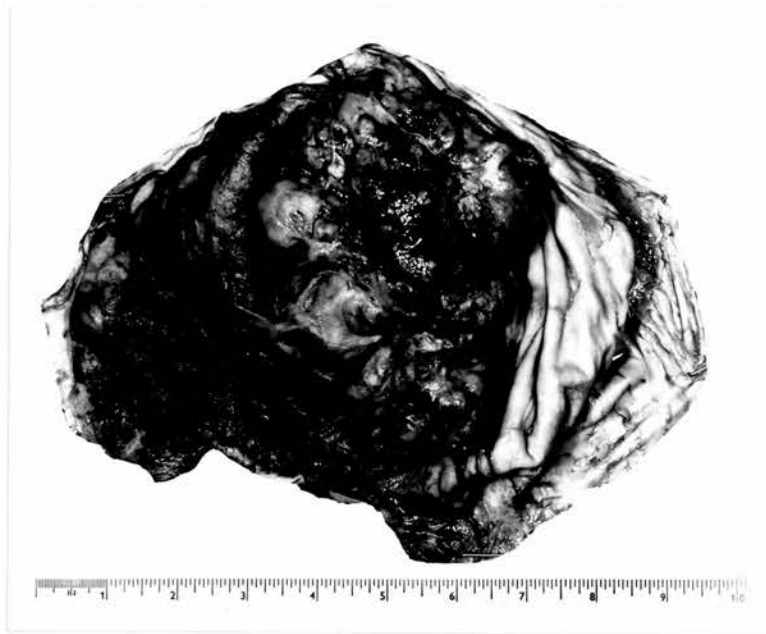


Fig. 30



Fig. 31

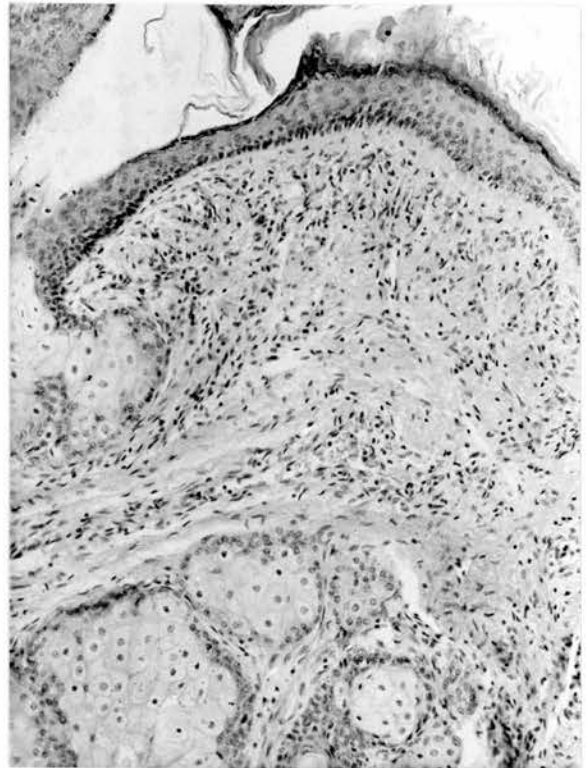


Fig. 32

Fig. 33 : Salivary glands and respiratory epithelium in the teratoma shown in the previous figures. X 40.

Fig. 34 : Nerve ganglion, seen in the muscle wall of a gut-like structure in the teratoma, indicative of a high degree of tissue maturation. X 63.

In spite of the maturity of the tissue elements of the teratoma, the prognosis should be guarded.

STRUMA OVARI

Thyroid tissue in the ovary is liable to the same functional and pathologic changes as thyroid tissue in the neck.

Fig. 35 : Struma ovarii showing the appearance of thyroid foetal adenoma. X 250.

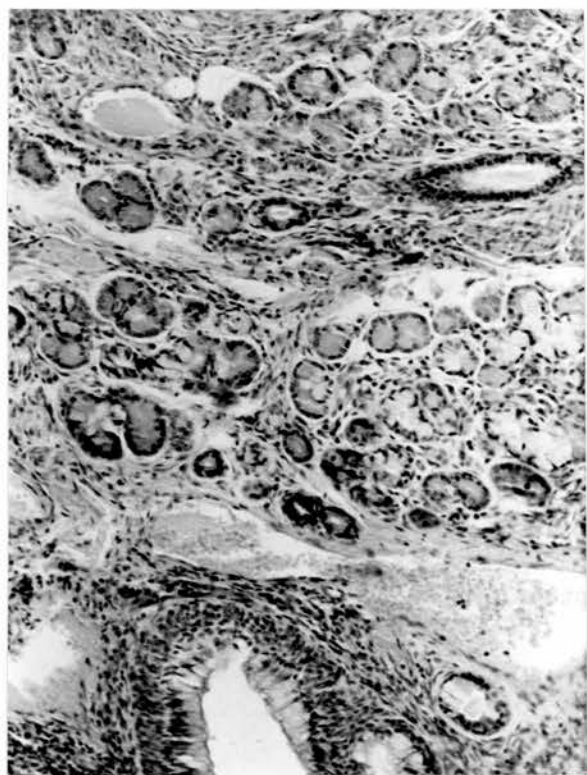


Fig. 33

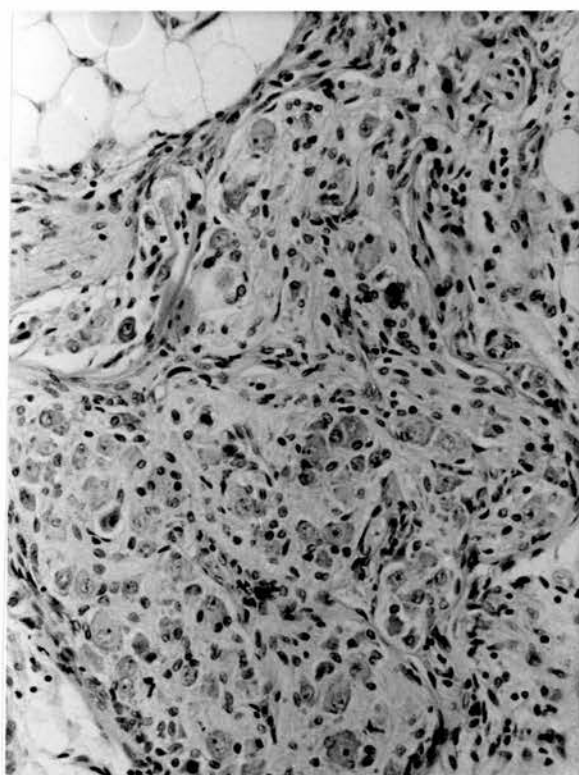


Fig. 34

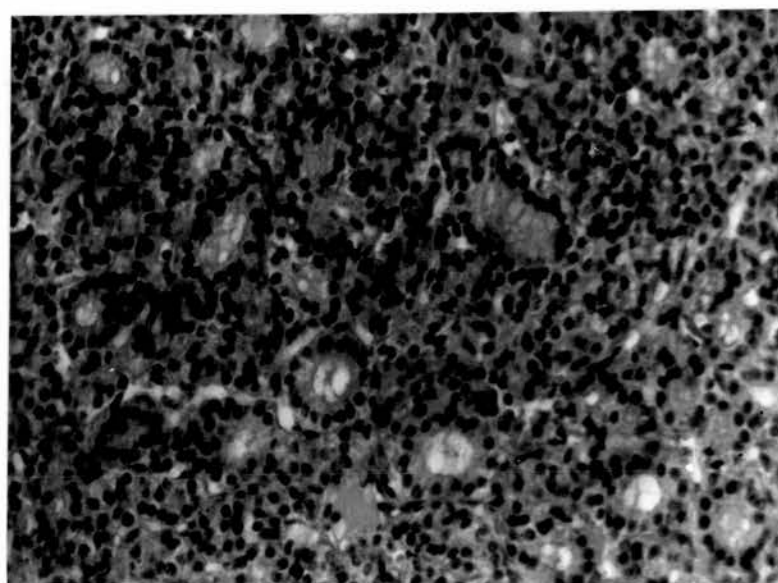


Fig. 35

BRENNER TUMOUR

In 18 out of 34 Brenner tumours, the ovary was normal-sized or only slightly enlarged.

Fig. 36 : Small Brenner tumour in the ovary, whole section. X 5.

Fig. 37 : Microscopical picture of the small Brenner tumour shown in the previous figure. X 65.

The sites of the small Brenner tumours may be of histogenetic significance.

Fig. 38 : A small cortical Brenner tumour. X 65.

An origin from rete structures is not applicable in this case.

Fig. 39 : A Brenner tumour in the hilum of the ovary. Neighbouring thick walled vessels in the hilum may be seen (left). X 100.

An origin from rete structures is possible.

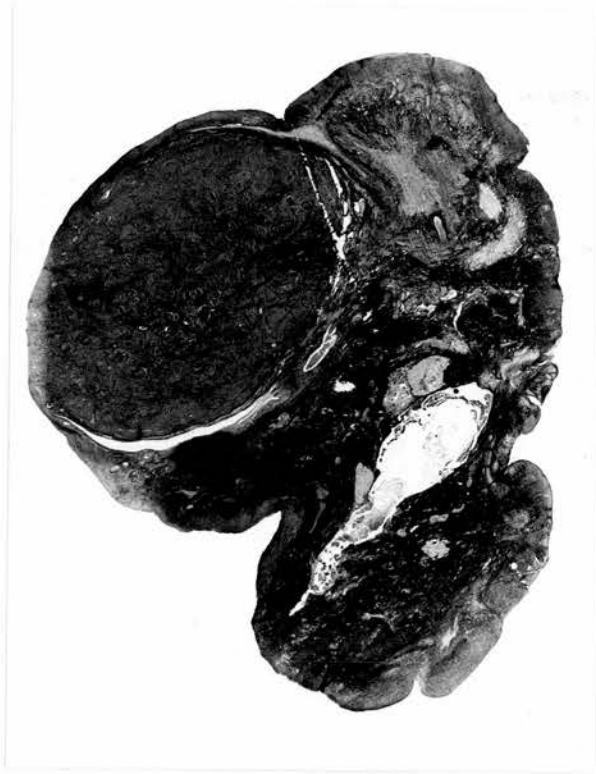


Fig. 36

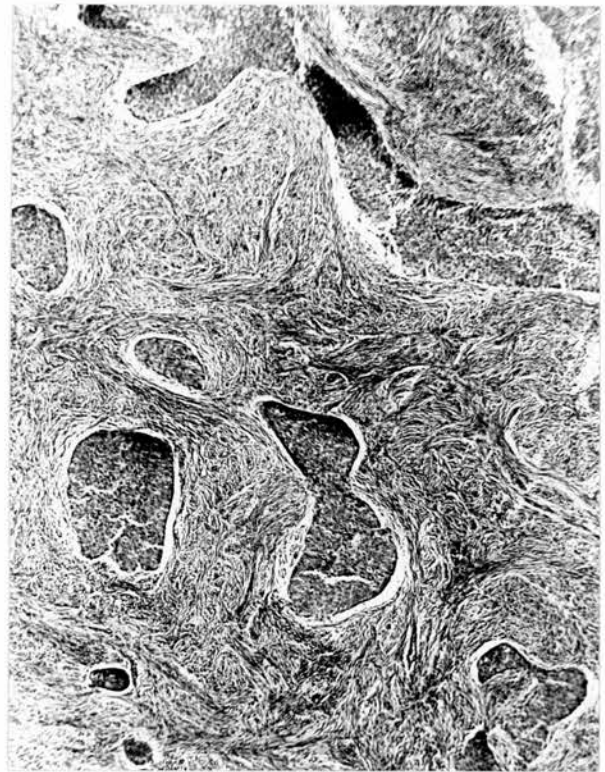


Fig. 37

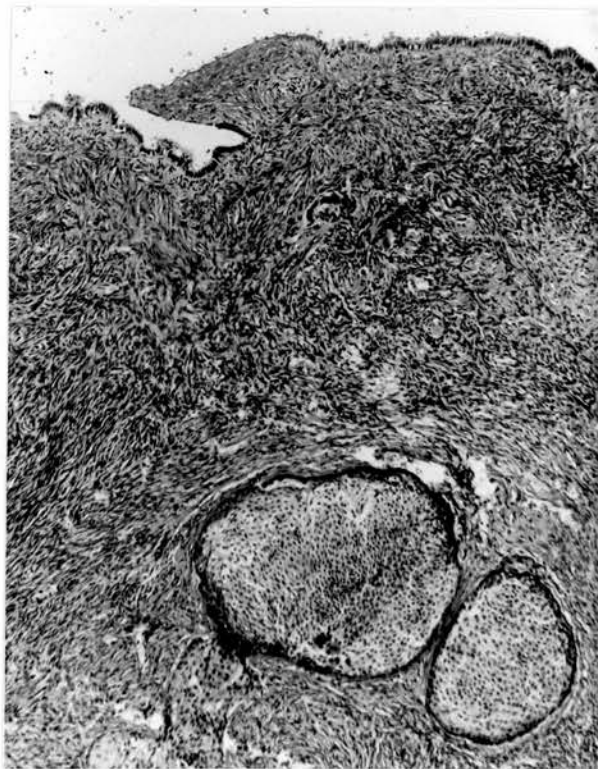


Fig. 38

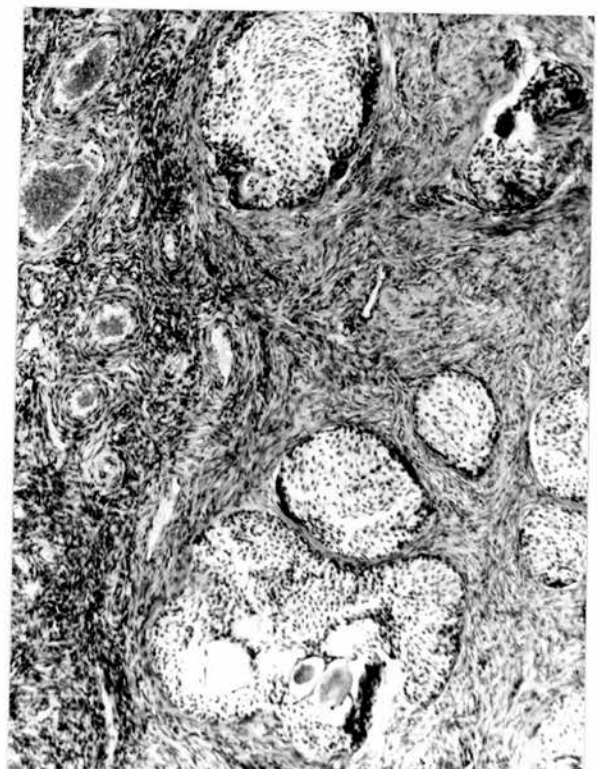


Fig. 39

ADENOFIBROMA AND CYSTADENOFIBROMA

In 15 out of 50 cases, the tumours produced little or no ovarian enlargement.

Fig. 40 and 41 illustrate such an example.

Fig. 40 : Whole section of the ovary showing the adenofibroma. X 5.

Fig. 41 : The same tumour under higher magnification. X 65.

Malignant transformation :

This condition is rare and may take one of several histologic types.

Fig. 42 : A borderline papillary lesion in a cystadenofibroma. The fibromatous part of the tumour is shown to the right. X 20.

Fig. 43 : Abnormal active proliferation of the stromal elements in another part of the same tumour shown in the previous figure (possibly not malignant). X 63.

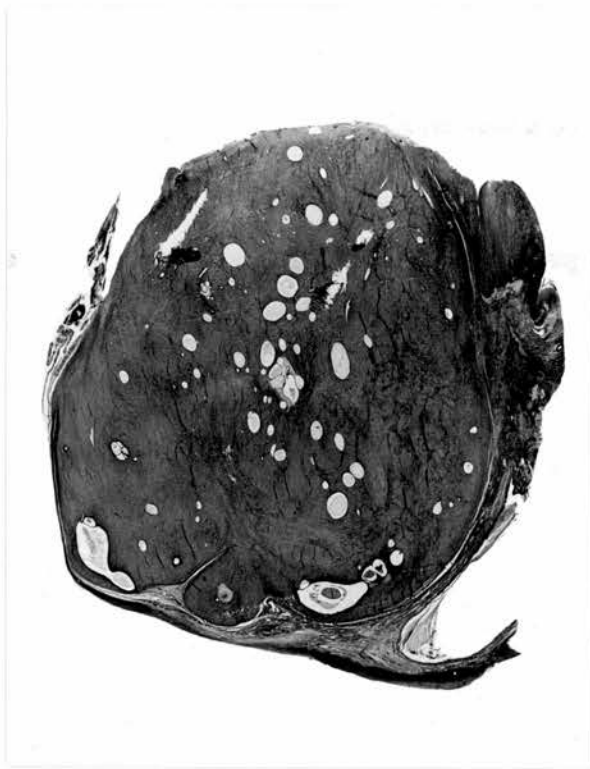


Fig. 40

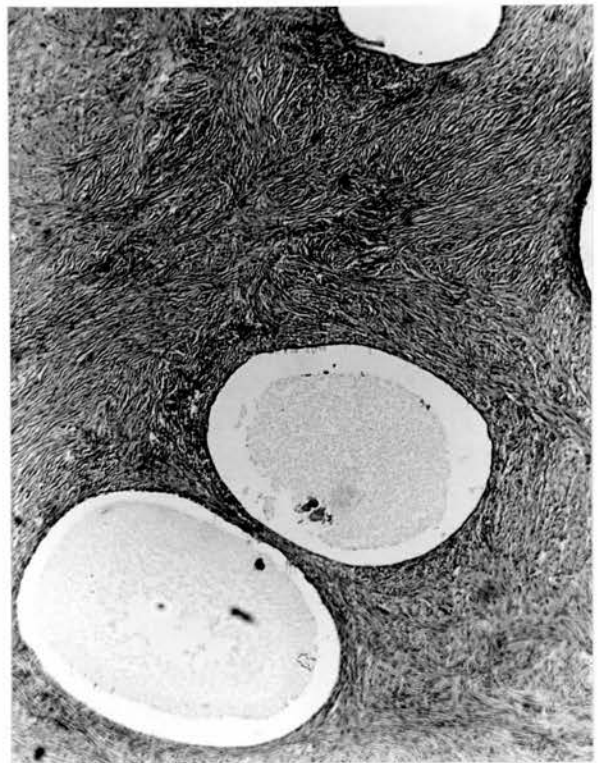


Fig. 41

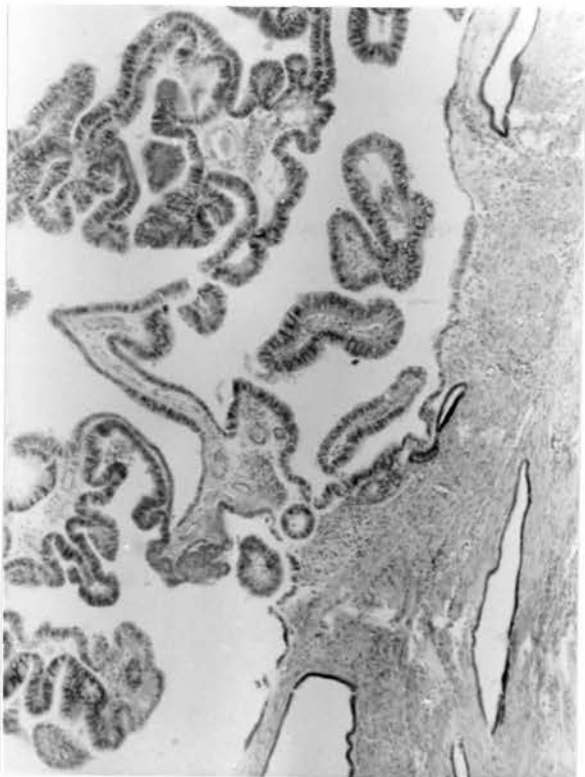


Fig. 42

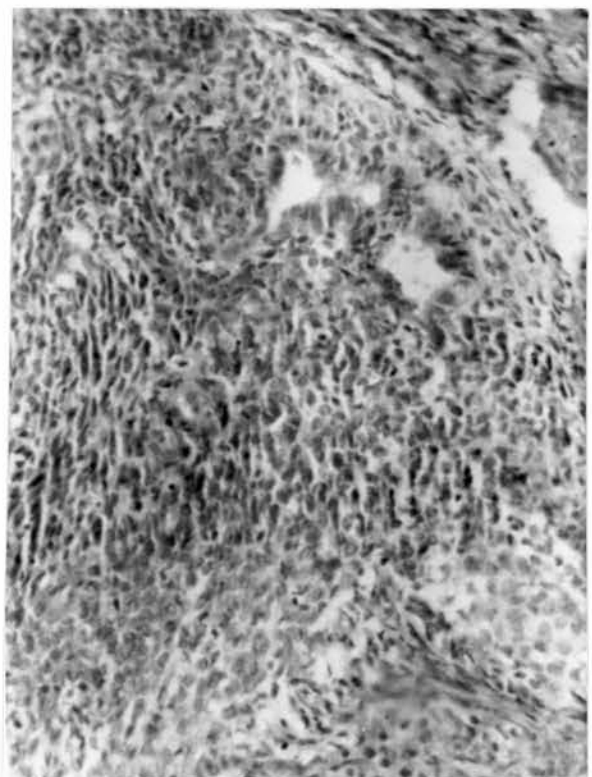


Fig. 43

Fig. 44 : An early polypoid adenocarcinoma in one of the locules of an otherwise benign cystadenofibroma. X 20.

Fig. 45 and 46 : A localized nodule of adenofibroma with acanthomatous change , in the wall of a benign mucinous cystadenoma.

Fig. 45 : Whole section photographed. The nodule is seen to the right and below. X 5.

Fig. 46 : Higher magnification of the adenofibroma showing the acanthomatous change. X 25.

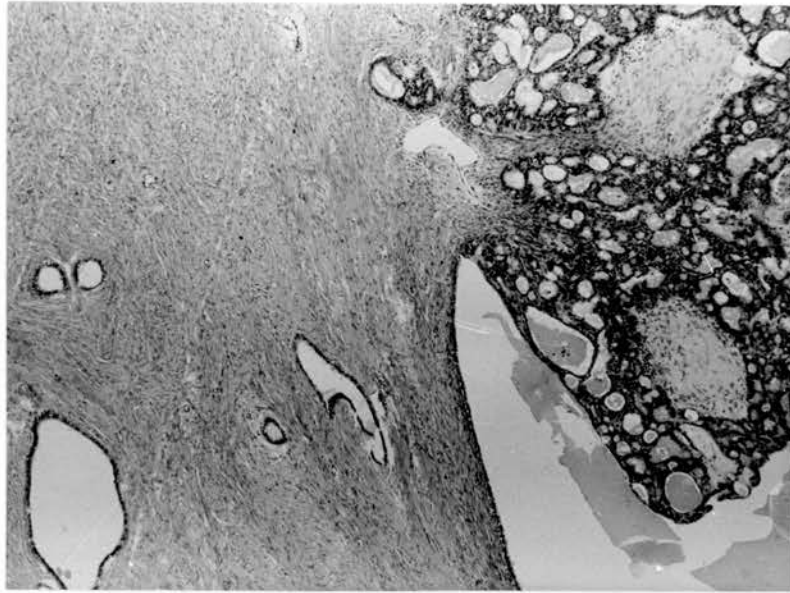


Fig. 44

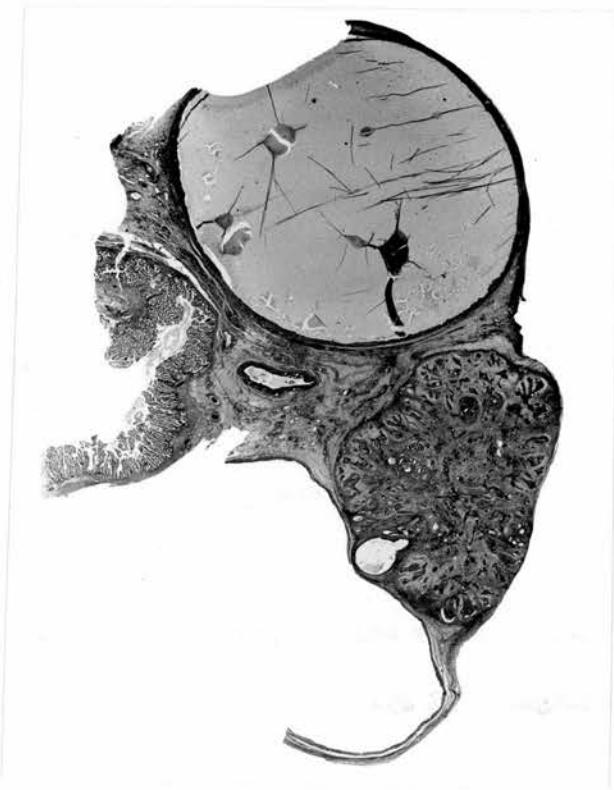


Fig. 45

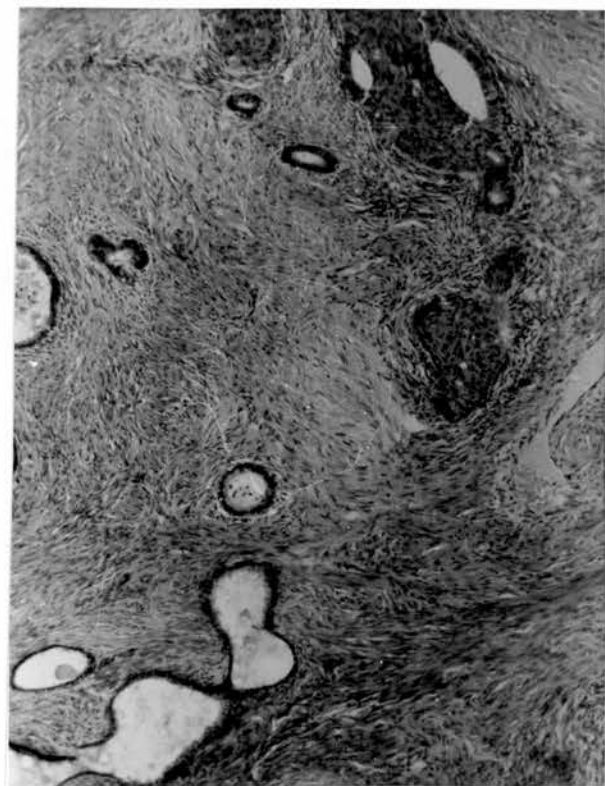


Fig. 46

Fig. 47 and 48 show an adenofibromatous area in the wall of a serous papillary cyst-adenocarcinoma and showing acanthomatous change.

Fig. 47 : The whole section photographed. X 5.

Fig. 48 : Higher magnification of the adenofibromatous area, illustrating the acanthomatous change. X 63.

This case and the previous one are probably examples of the multiple Mullerian potentialities of the surface epithelium of the ovary.

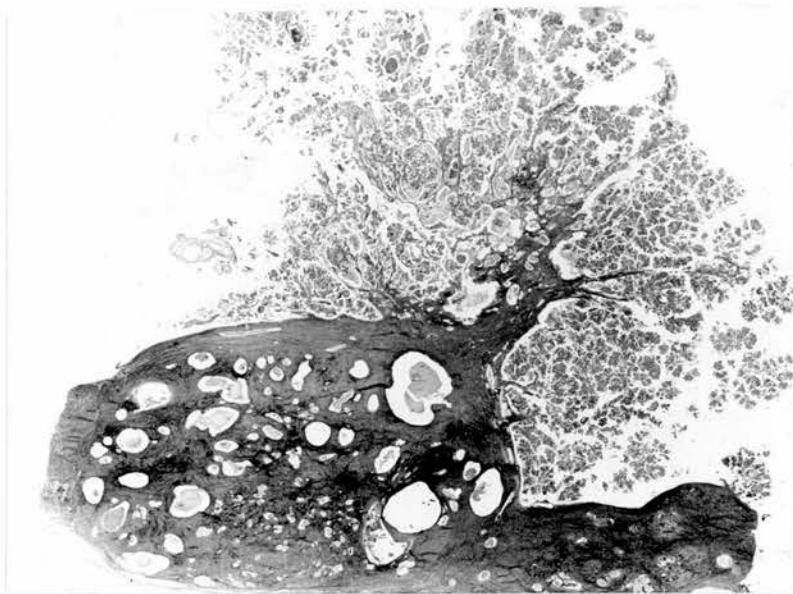


Fig. 47

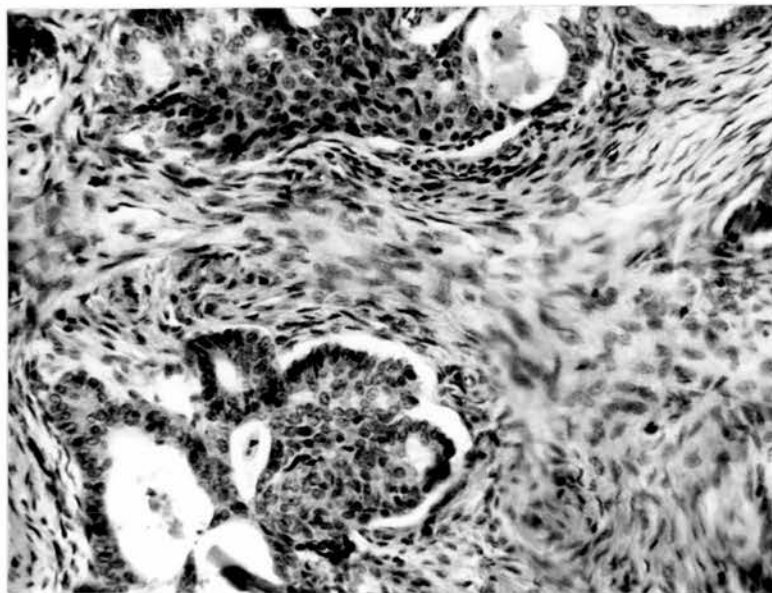


Fig. 48

FIBROMA

Out of 135 cases of ovarian fibroma, 29 were encountered in normal-sized ovaries.

Fig. 49 and 50 illustrate such an example.

Fig. 49 : Whole section of ovary, showing fibroma. X 5.

Fig. 50 : The fibroma, at a higher magnification. X 63.

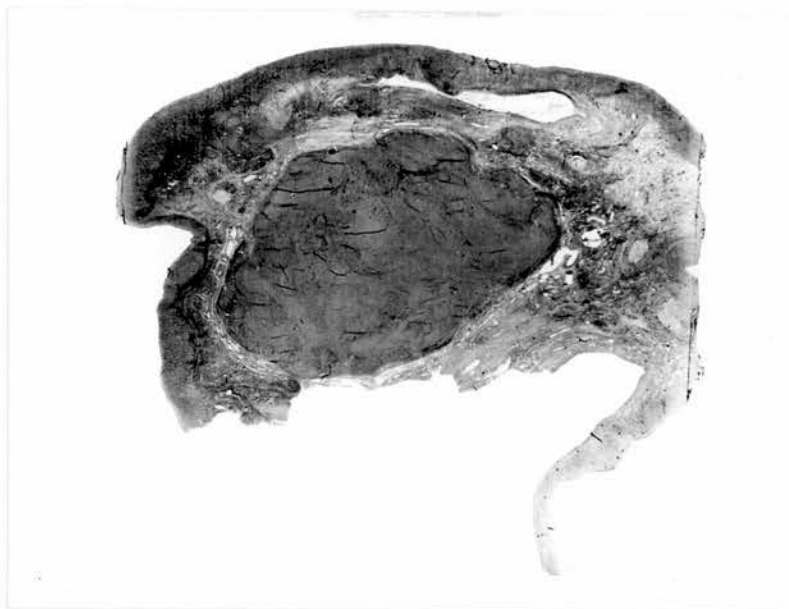


Fig. 49

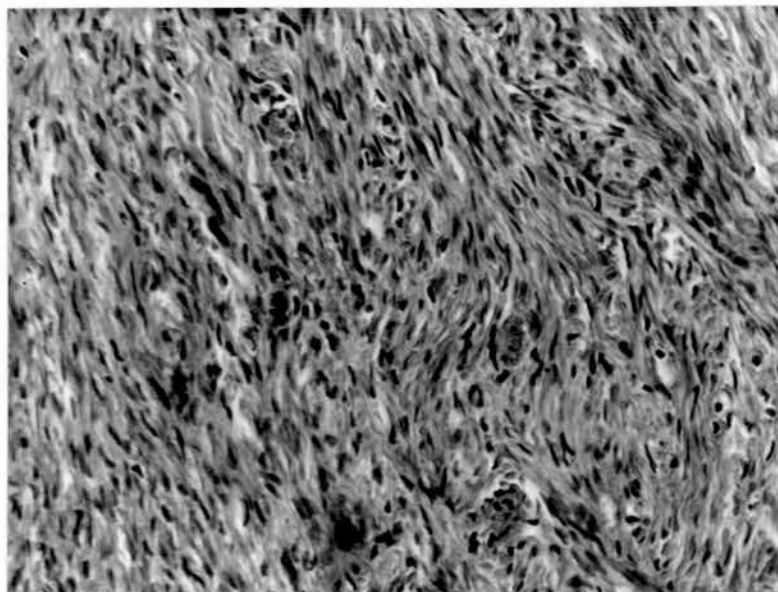


Fig. 50

ARRHENOBLASTOMA AND GRANULOSA CELL TUMOUR

Although it has been customary to regard the arrhenoblastoma and the granulosa cell tumour as tumour types which are sharply distinct in their morphology, histogenesis as well as in their endocrine potential, this distinction does not seem now to be so exact.

In tumours of apparently granulosa cell type , a picture suggestive of arrhenoblastoma may be seen in some areas of the tumour. Fig. 51 and 52 illustrate such an example. Fig. 51 : A picture typical of granulosa cell tumour. Rosettes may be seen.

X 255.

Fig. 52 : Another area in the same tumour suggests the pattern of testicular tubular adenoma. Compare with figure 135. X 255

In some tumours, especially if evidence of hormonal activity ~~was~~ lacking, it may be impossible to distinguish a granulosa cell tumour from an arrhenoblastoma. Two examples are illustrated here.

Fig. 53 : Granulosa cell tumour (middle) or arrhenoblastoma (sides) ?.

No clinical endocrine manifestations were present. X 63.

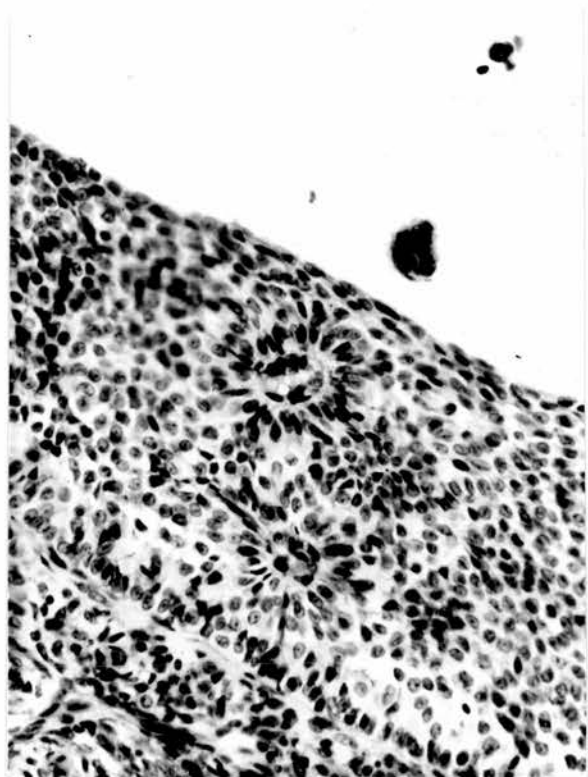


Fig. 51

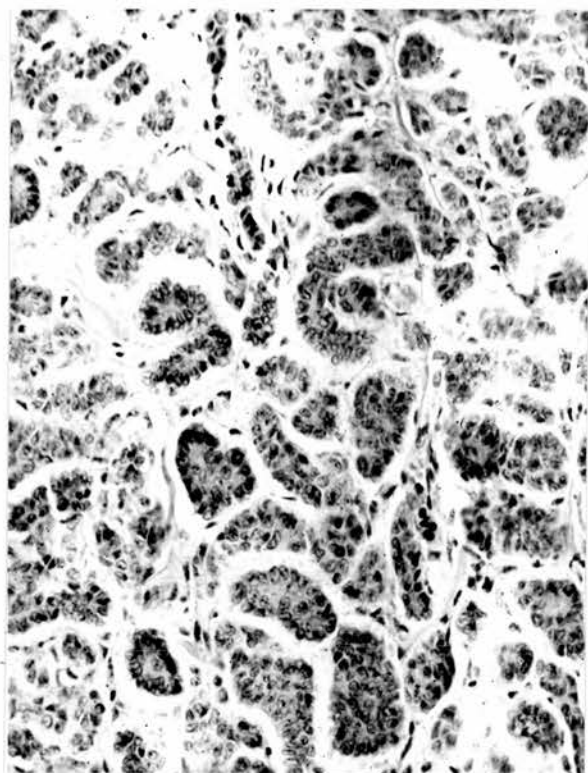


Fig. 52

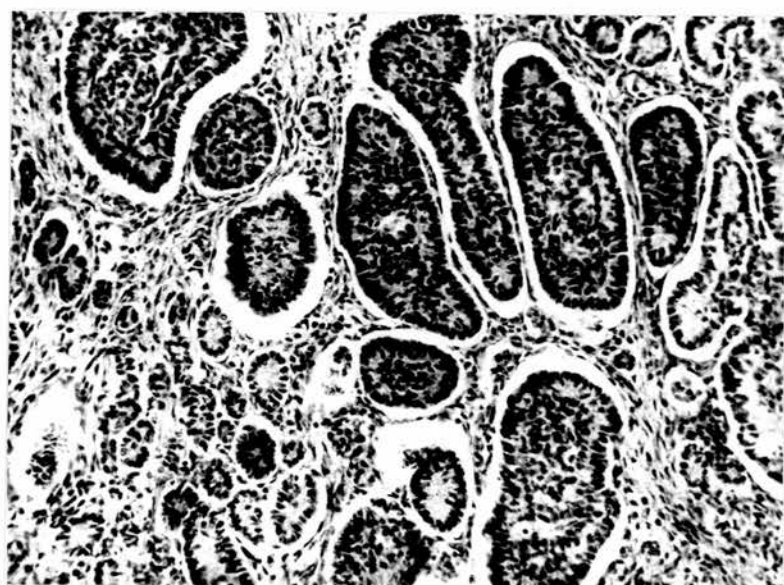


Fig. 53

Fig. 54 : Granulosa cell tumour or arrhenoblastoma ?. No clinical endocrine manifestations were present. The picture is that of a sex cord (Granulosa or Sertoli) mesenchymal (Theca or Leydig) tumour. X 40.

Fig. 55 : Arrhenoblastoma, associated with clinical masculinization. Masculinizing arrhenoblastomas rarely show a resemblance to testicular tissue. Note the abundant Leydig cells in the tumour. X 175.



Fig. 54

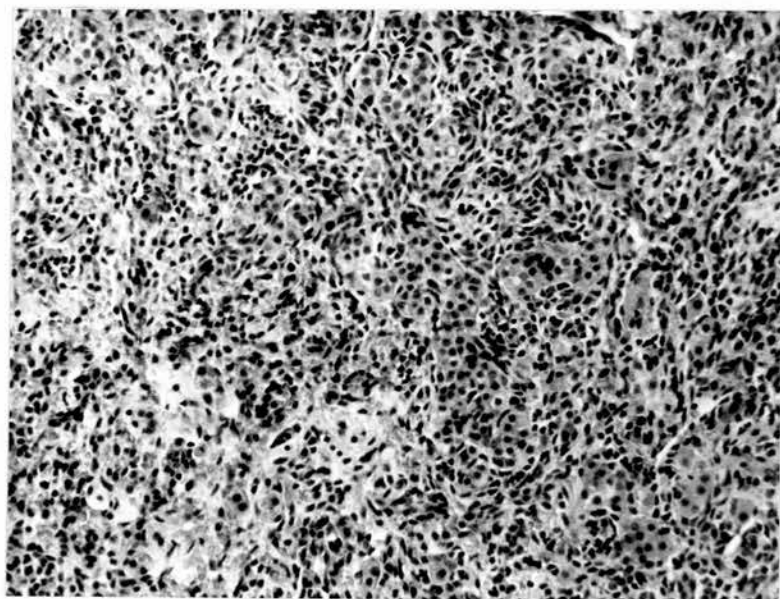


Fig. 55

LIPOID CELL TUMOURS

Fig. 56, 57 and 58 show an oestrogenic hilus cell tumour.

Fig. 56 : Section of whole ovary and the tumour. X 5.

Fig. 57 : The tumour at a higher magnification. X 63.

Fig. 58 : The postmenopausal endometrium showing cystic hyperplasia. X 110.



Fig. 56

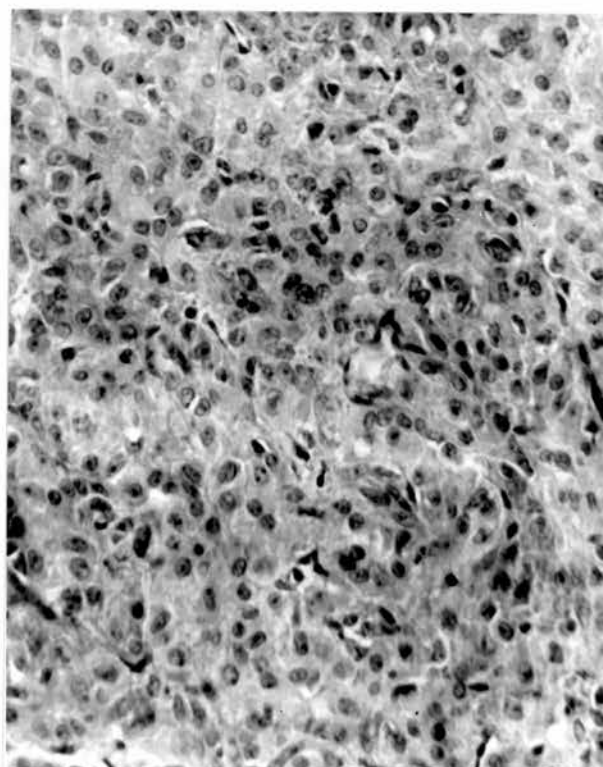


Fig. 57



Fig. 58

Fig. 59 : An adenoma of hilus cells in the hilum of the ovary. (The patient was post-menopausal and the endometrium showed cystic hyperplasia) . X 40.

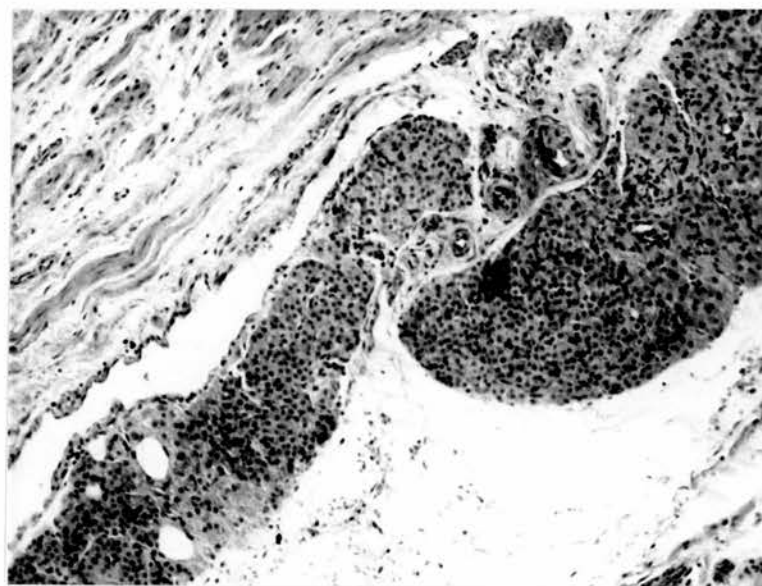


Fig. 59

Fig. 60, 61 and 62 illustrate a lipoid cell tumour, associated with no clinical evidence of hormonal activity.

Fig. 60 : Whole section of ovary and tumour (nodule at top).

X 5.

Fig. 61 : Microscopical appearance of the tumour. X 40.

Fig. 62 : Atrophic postmenopausal endometrium. X 110.

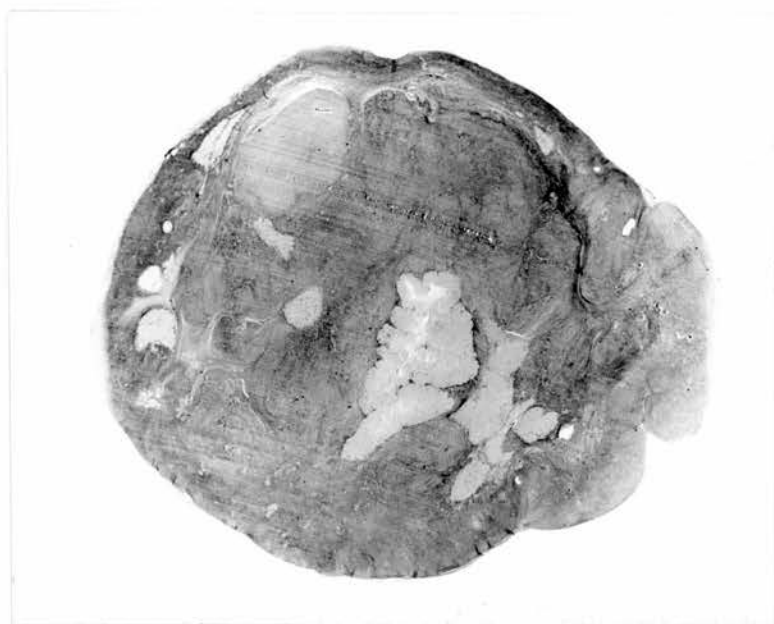


Fig. 60

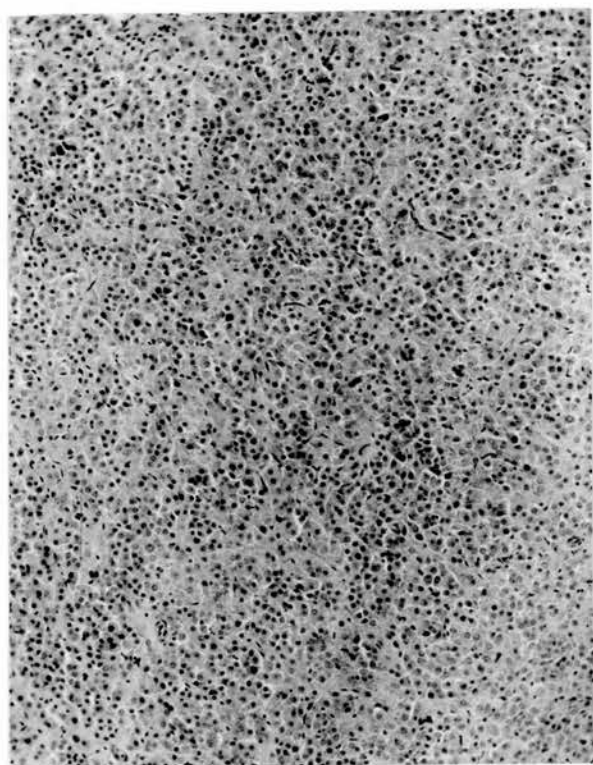


Fig. 61

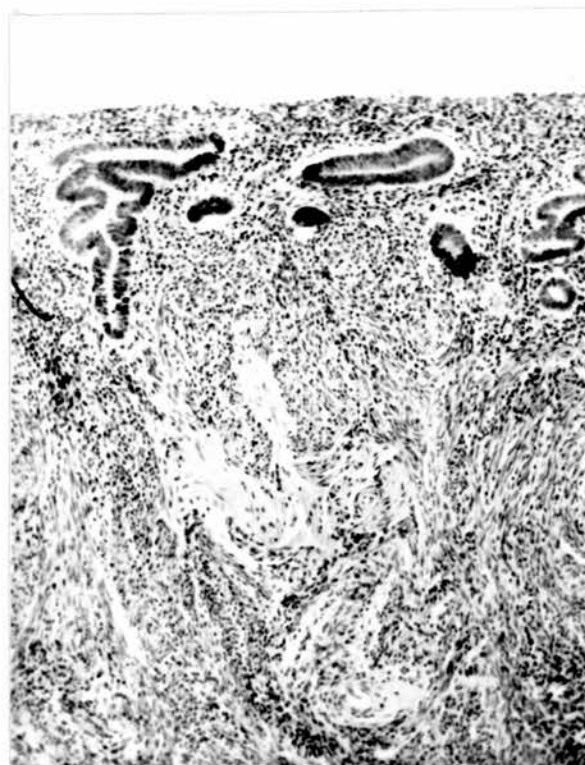


Fig. 62

THE ORIGIN OF MALIGNANT OVARIAN TUMOURS IN ENDOMETRIOSIS

Fig. 63 : Atypical epithelium in an endometrial cyst.

Note the heaping up and the cellular and nuclear pleomorphism.

No definite evidence of malignancy with stromal invasion. X63.

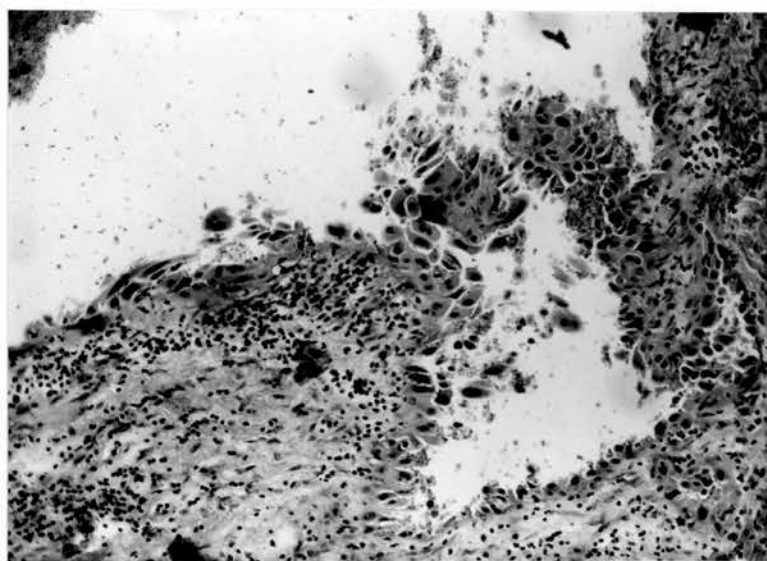


Fig. 63

Fig. 64 : Ovarian carcinoma arising in endometriosis. Case 1.

An early polypoid malignancy in an ovarian endometrial cyst. X 5.

Fig. 65 : Ovarian carcinoma arising in endometriosis. Case 1.

Higher magnification of the previous figure, to show the transition between benign and malignant endometrium. X 30.



Fig. 64



Fig. 65

Fig. 66 : Ovarian carcinoma arising in endometriosis. Case 1.

Cyst excised 18 months after removal of the tumour shown in the previous two figures : A broad zone of pseudo-xanthoma cells, suggestive of endometriosis, is seen lining the cyst. The ovarian carcinoma is seen above. X 105.

Fig. 67 : Ovarian carcinoma arising in endometriosis. Case 1.

Tumour excised 18 months after removal of the tumour shown in figure 64 and 65 : Adenoacanthoma showing the malignant character of the squamous as well as the glandular component. X 170.

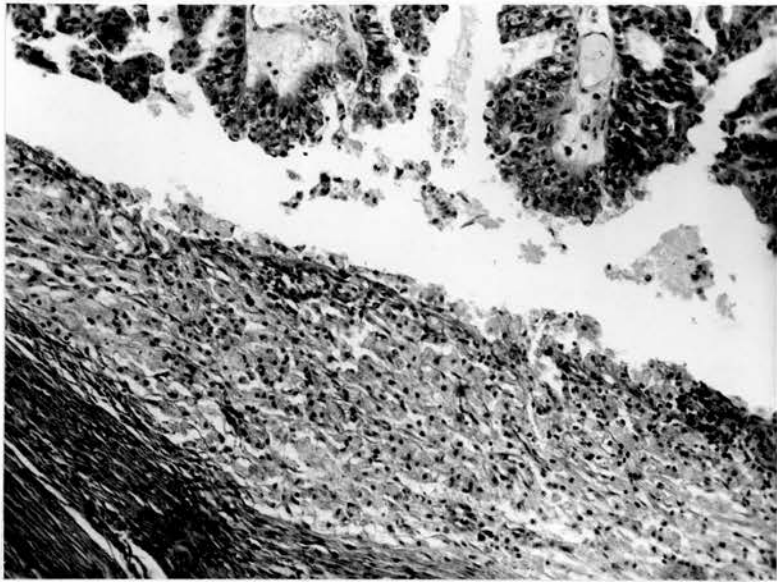


Fig. 66

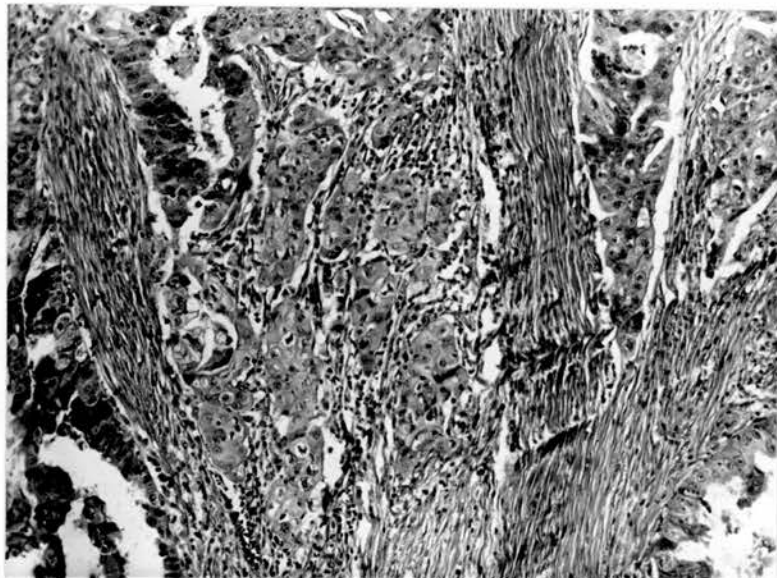


Fig. 67

Fig. 68 : Ovarian carcinoma arising in endometriosis. Case 2.

A localized papillary adenoacanthoma . X 105.

Fig. 69 : Ovarian carcinoma arising in endometriosis. Case 3.

Endometrial cyst with two small polypoid malignant growths and an area of
endometrial cystic hyperplasia (top left). X 5.

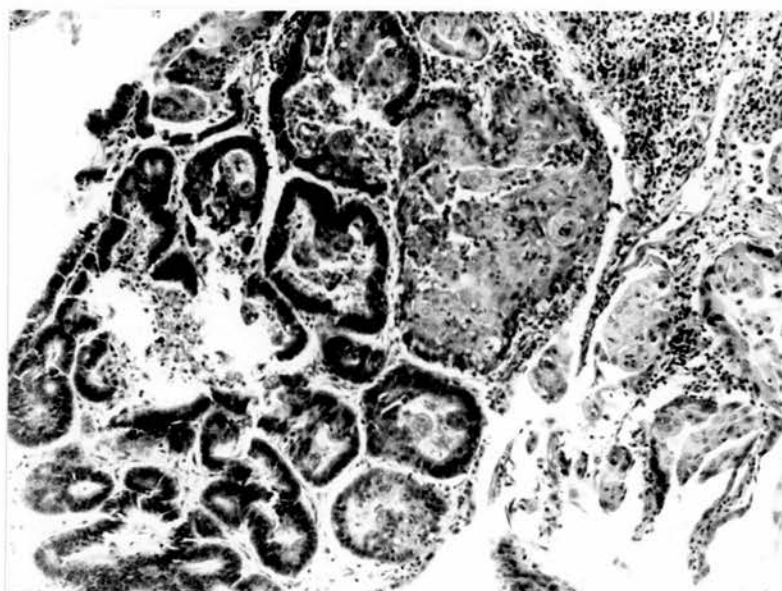


Fig. 68

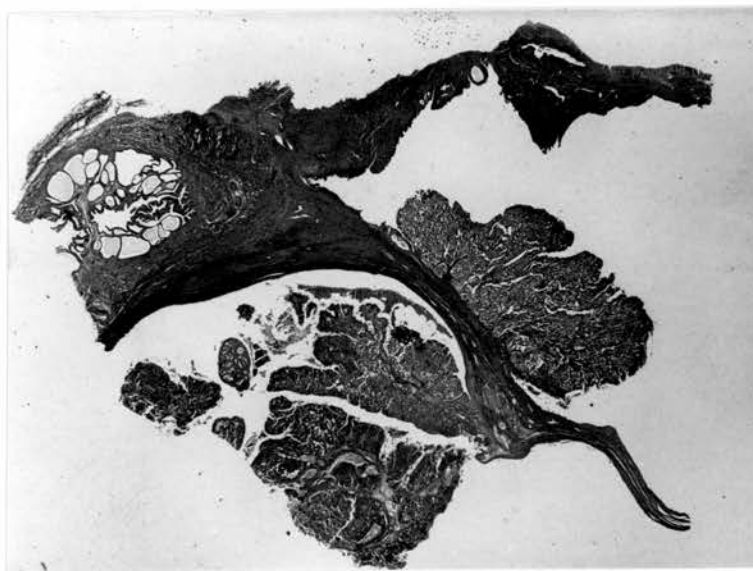


Fig 69

Fig. 70 : Ovarian carcinoma arising in endometriosis. Case 4.

Papillary carcinoma arising into and continuous with the benign epithelial lining of an endometrial cyst. X 30.

Fig. 71 : Ovarian carcinoma arising in endometriosis. Case 4.

Higher magnification of the carcinoma, to show its endometrioid character.

X 60.



Fig. 70

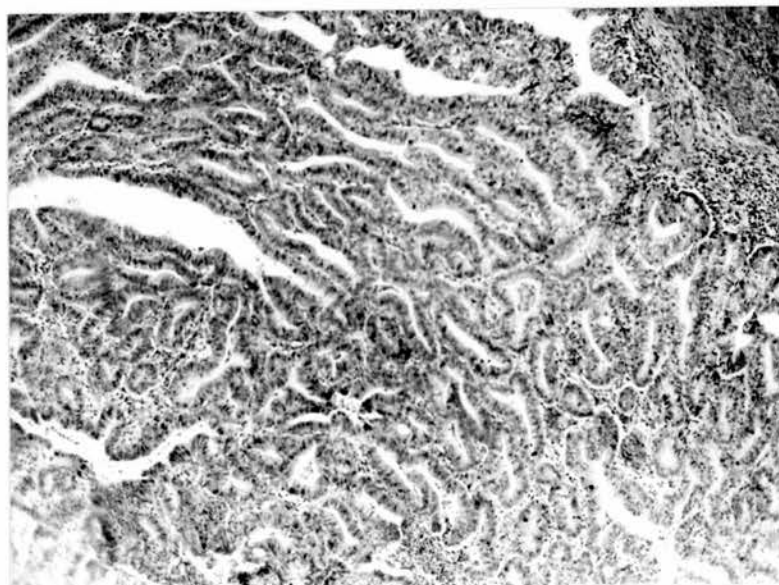


Fig. 71

Fig. 72 : Ovarian adenocarcinoma showing acanthomatous change.

Origin in endometriosis could not be demonstrated but cannot be ruled out.

X 90.

Fig. 73 and 74 show the association of ovarian adenoacanthoma and squamous metaplasia in the uterine endometrium.

Fig. 73

Primary ovarian adenoacanthoma. X 100

Fig. 74

Squamous metaplasia in the benign proliferative uterine endometrium.
X 90.

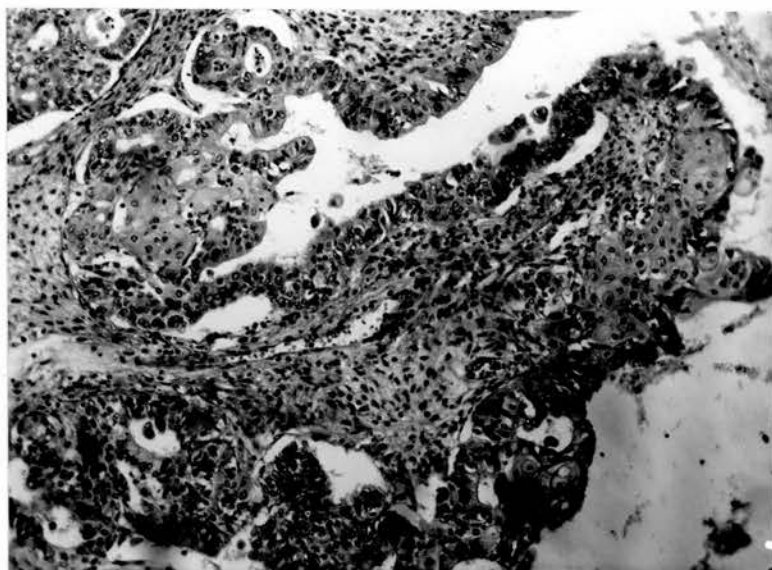


Fig. 72



Fig. 73

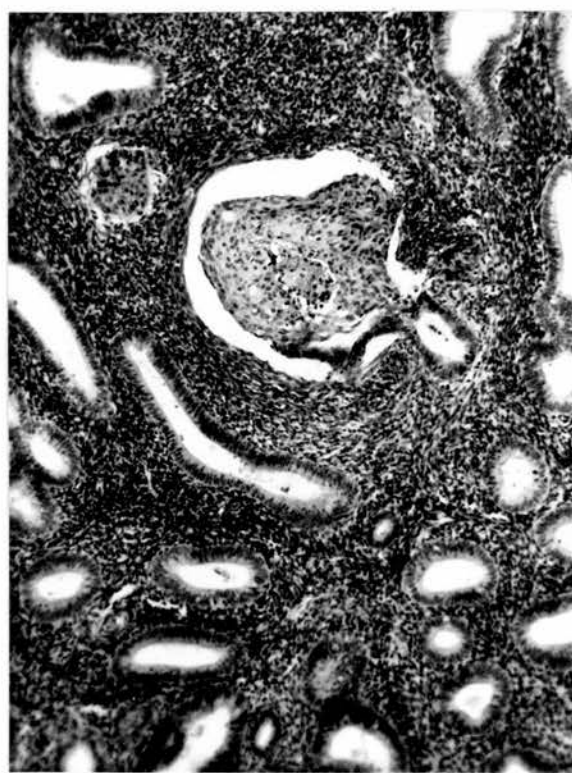


Fig. 74

THE OCCURRENCE OF GRANULOSA AND THECA CELL TUMOURS IN CLINICALLY NORMAL OVARIES

Fig. 75, 76, 77 and 78 :

The history of this patient spanned over three and half years, with irregular bouts of postmenopausal bleeding. The uterus was curetted three times before total hysterectomy with bilateral oophorectomy was at last decided upon.

Fig. 75 : Whole section of ovary and tumour. X 5.

Fig. 76 : Microscopical appearance of the tumour showing a granulosa cell picture.
X 180.

Fig. 77 : The postmenopausal endometrium showing cystic hyperplasia. X 65.

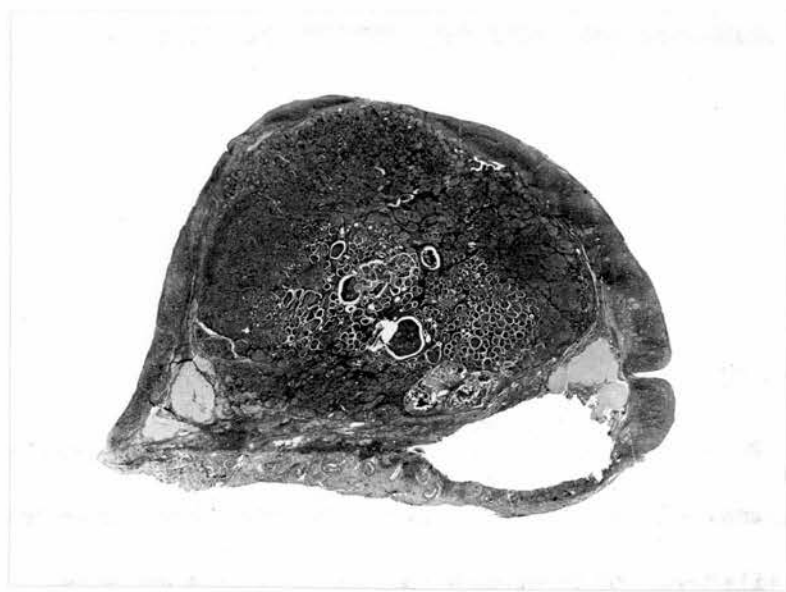


Fig. 75



Fig. 76

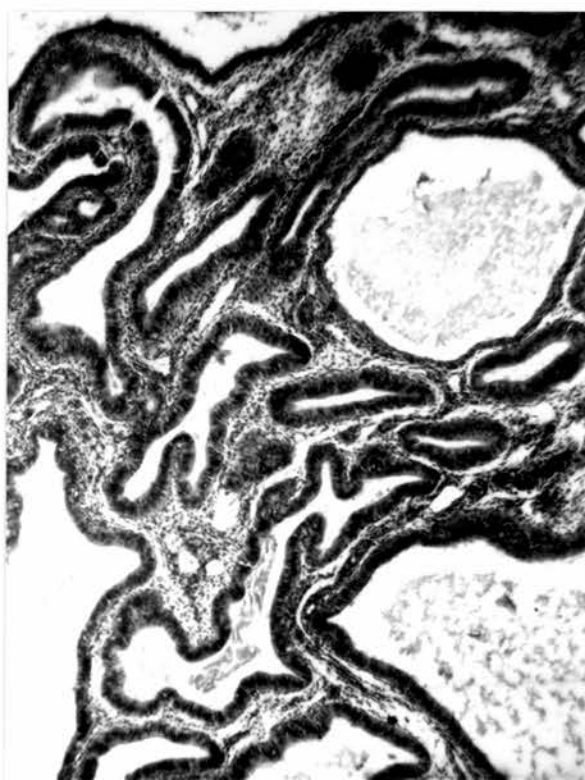


Fig. 77

Fig. 78 : Corpus-albicans-like structure with granulosa cells , at the periphery of
the granulosa cell tumour shown in figure 75 and 76. X 175.

The origin of granulosa cell tumours in atretic follicles has been suggested.

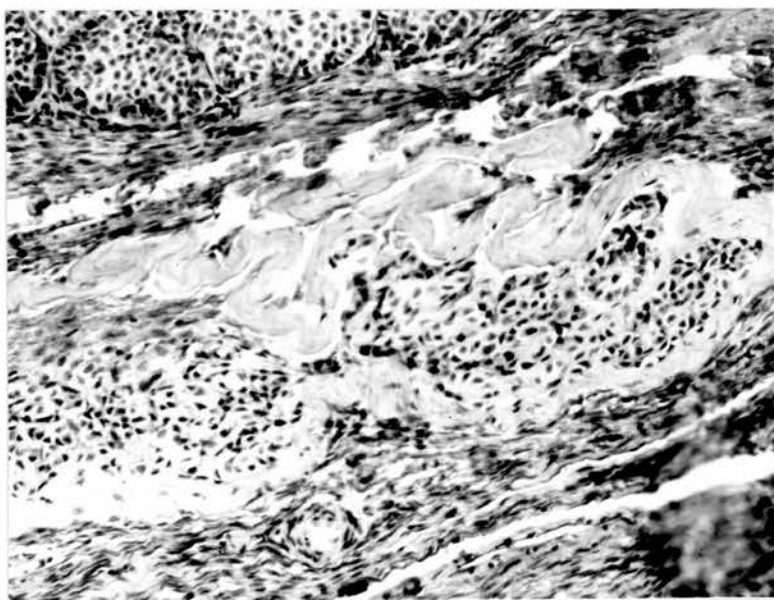


Fig. 78

Fig. 79, 80 and 81 :

The patient , aged 58 years and postmenopausal for 5 years, suffered from attacks of uterine bleeding during the last three years. Uterine curettage was performed twice during this period and showed cystic hyperplasia. Laparotomy was performed after the second curettage.

Fig. 79 : Whole section of ovary and tumour. X 5.

Fig. 80 : Microscopical appearance of the tumour, showing a theca cell picture. Luteinization was prominent, a feature commonly observed in these small tumours. X 180.

Fig. 81 : The postmenopausal endometrium showing cystic hyperplasia. X 65.



Fig. 79

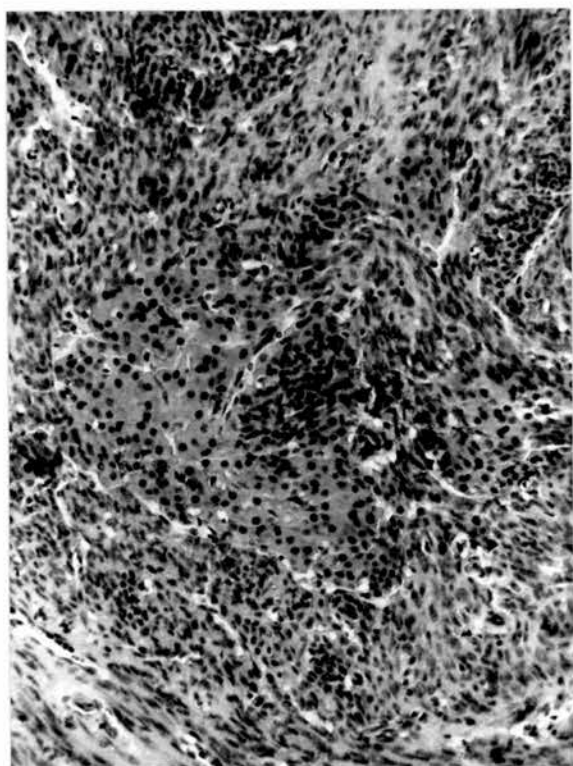


Fig. 80

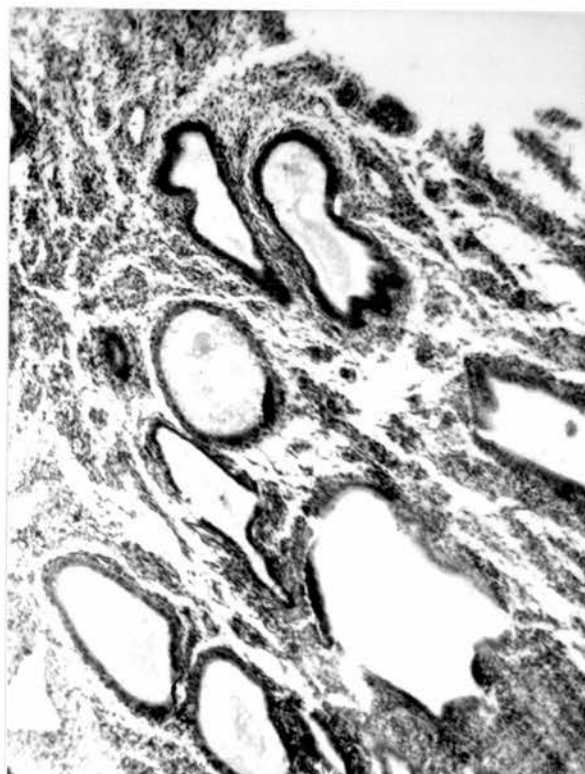


Fig. 81

Fig. 82 and 83 :

The granulosa cell tumour in this case was an accidental finding. A large solid adenocarcinoma was present in the opposite ovary. The endometrium was inactive.

Fig. 82 : Whole section of ovary and tumour. X 5.

Fig. 83 : Microscopical appearance of the tumour, showing a granulosa cell picture. X 63.

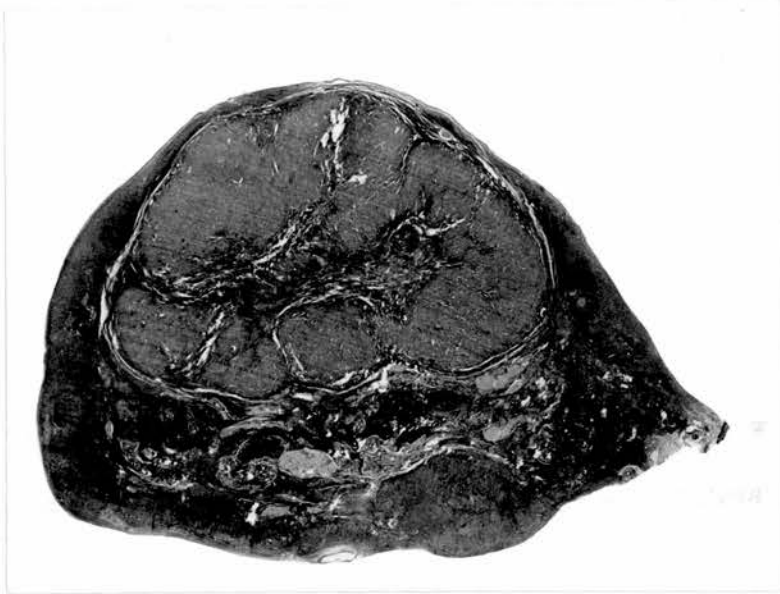


Fig. 82

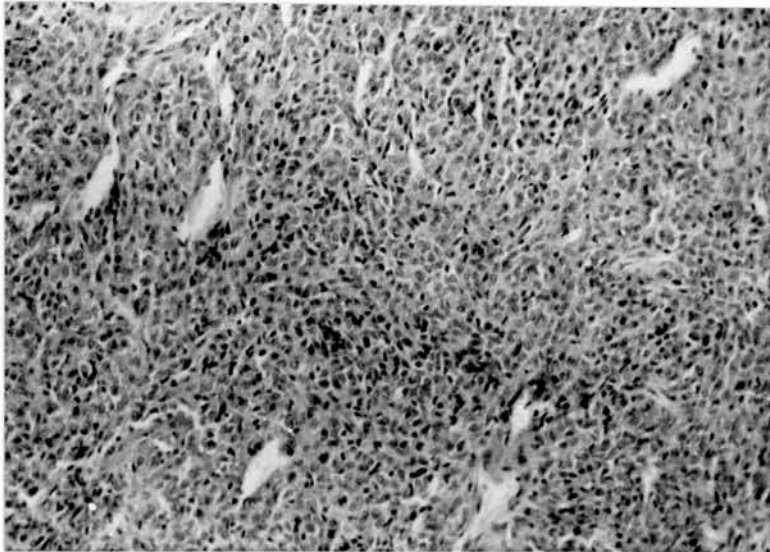


Fig. 83

Fig. 84 and 85 :

The patient, aged 57 years and 7 years postmenopausal, presented with irregular uterine bleeding of 15 months duration. Endometrial curettings showed cystic hyperplasia and laparotomy was performed.

Fig. 84 : Whole section of ovary and tumour. X 5.

Fig. 85 : Microscopical picture showing a luteinized theca cell tumour. X 40.

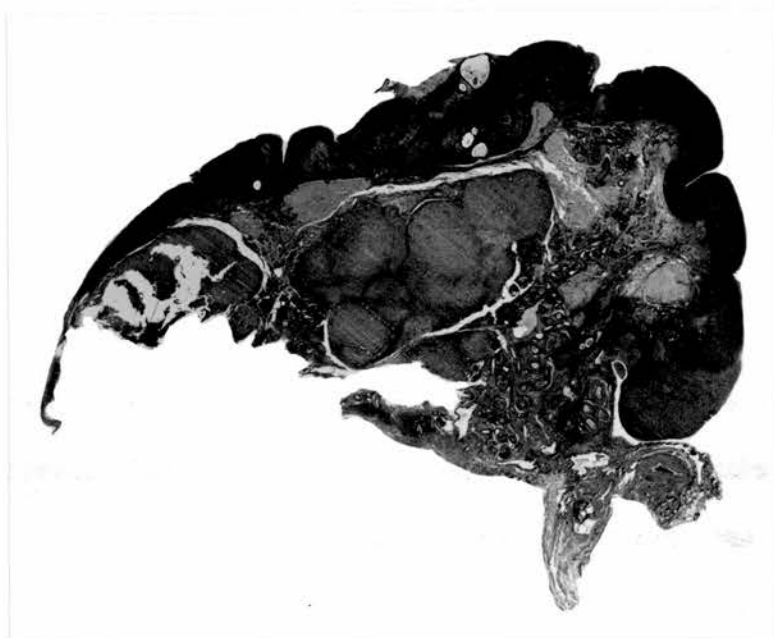


Fig. 84

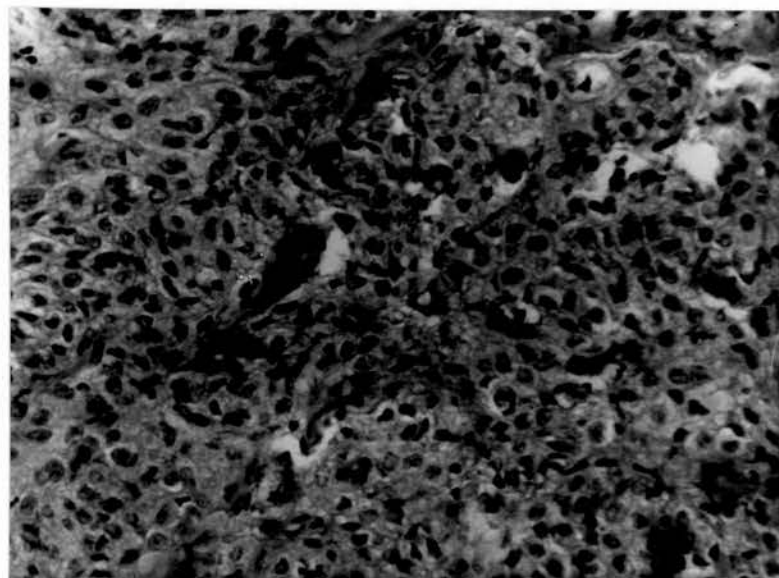


Fig. 85

Fig. 86 and 87 :

The patient, aged 56 years, presented with bleeding per vaginum of two years duration and gradually increasing in severity. Endometrial curettings showed active cystic hyperplasia with some areas showing an irregular adenomatous pattern. Total hysterectomy with bilateral salpingo-oophorectomy was performed. After operation, the patient suffered from hot flushes which passed off gradually. The patient, 8 years later, was living and well, though complaining of senile vaginitis.

Fig. 86 : Whole section of ovary and tumour. X 5.

Fig. 87 : Microscopical appearance showing the picture of a theca cell

tumour with prominent luteinization, a feature commonly observed in these small tumours. X 40.

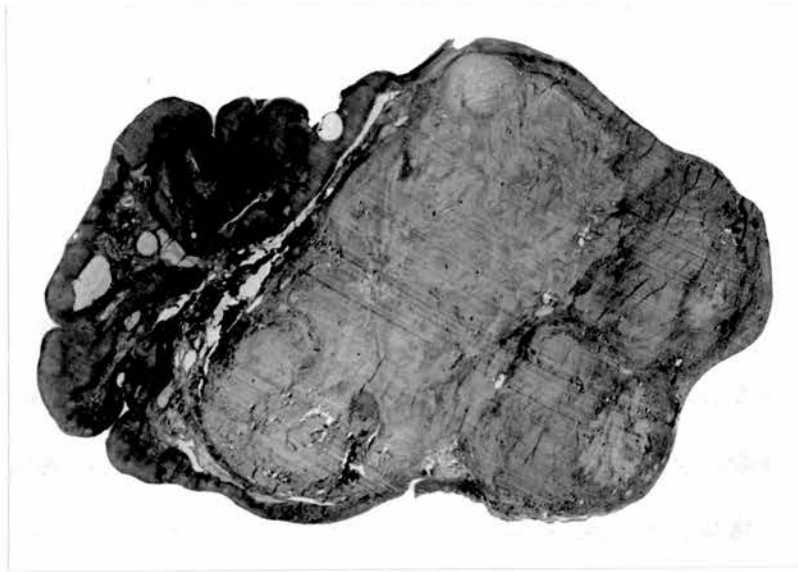


Fig. 86

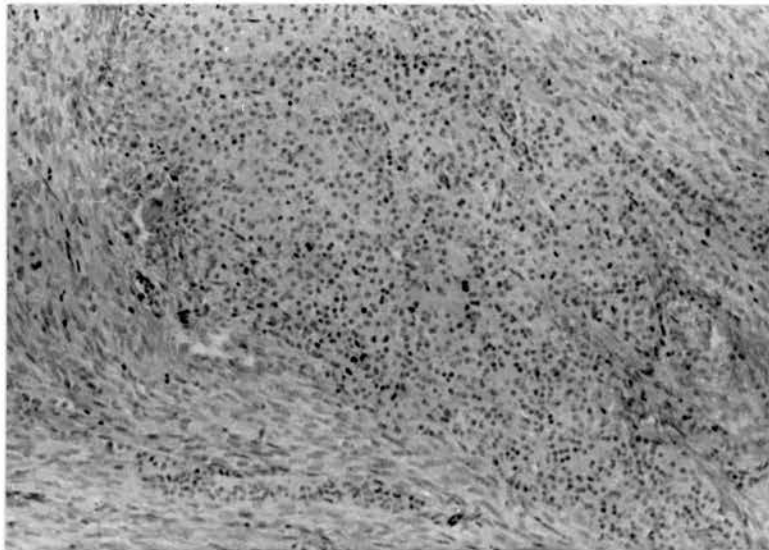


Fig. 87

Fig. 88, 89, 90 and 91 :

This patient was first seen at the age of 65 years, 15 years after the onset of the menopause. She had vaginal bleeding for 4 days and also complained of genital prolapse. A Fothergill operation was performed. Uterine curettings showed an active endometrium as well as a benign polyp. Bleeding recurred after 8 months and a second curettage showed endometrial hyperplasia and another polyp. Ten months later, bleeding recurred and the third curettage was performed and showed a hyperplastic cystic endometrium with adenomatous areas. One year and three months later, the patient presented with another attack of bleeding. Total hysterectomy with bilateral salpingo-oophorectomy was performed.

Fig. 88 : Whole section of ovary and tumour. X 5.

Fig. 89 : Microscopical appearance of the tumour, showing a theca cell picture with prominent luteinization, a feature that was commonly noted in these small tumours. X 100

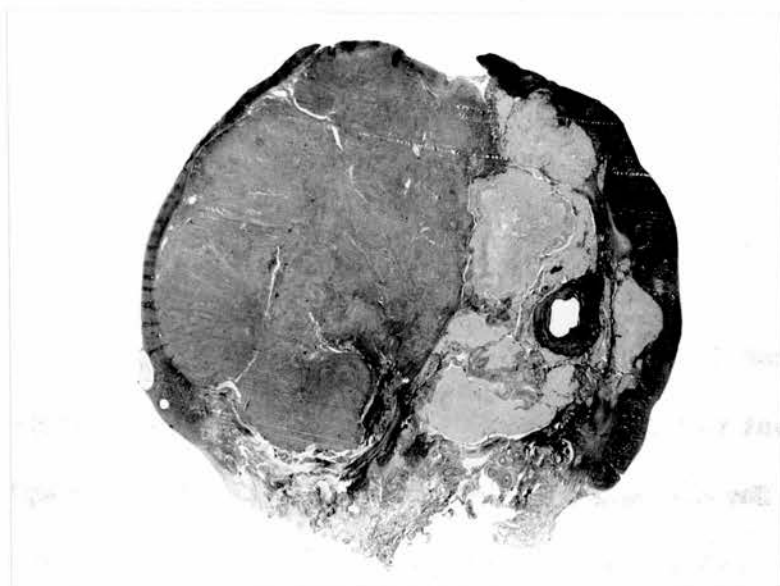


Fig. 88

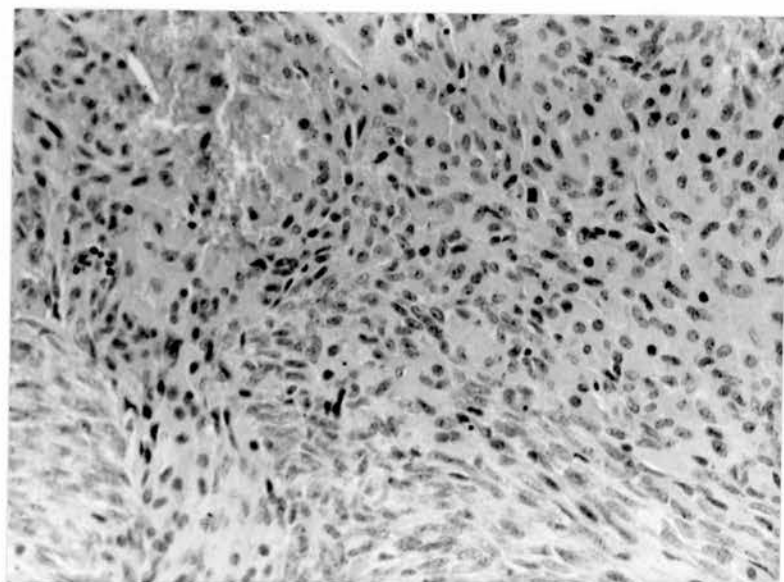


Fig. 89

Fig. 90 : Postmenopausal endometrial cystic hyperplasia, second curetting. X 20.

Fig. 91 : The endometrium , two years later, also showing cystic hyperplasia. X 20.

Full notes in previous page.

Fig. 92 : Hyperplasia of the ovarian stroma with foci of luteinization. The ovary contained a small theca cell tumour. X 180.

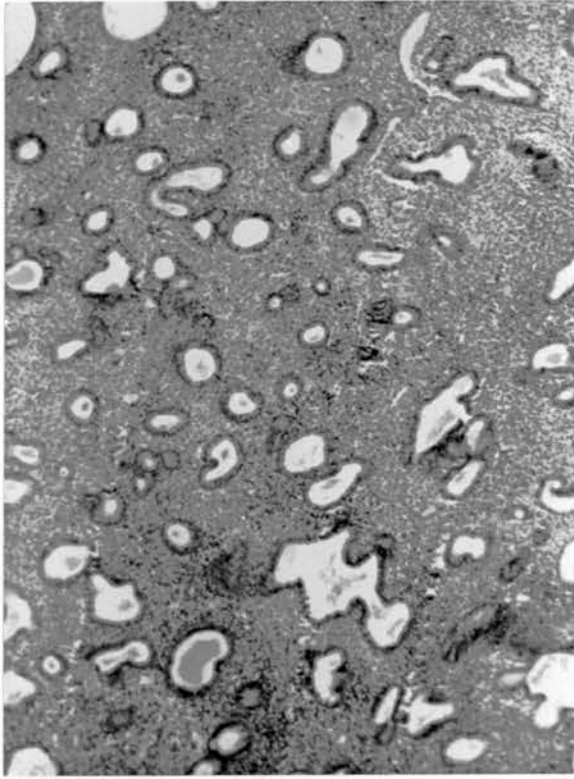


Fig. 90

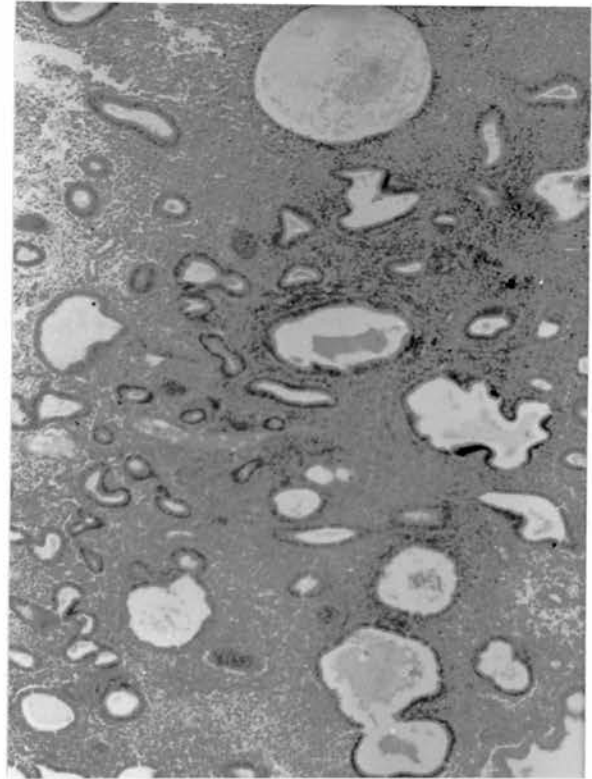


Fig. 91

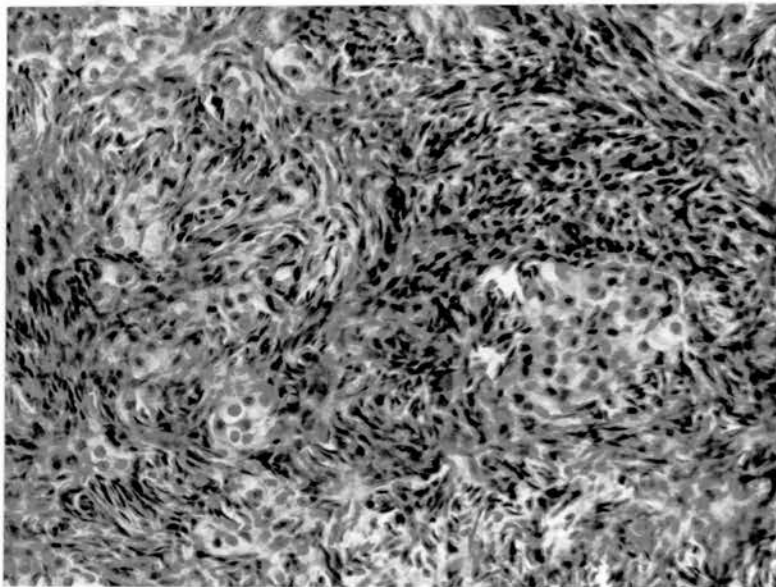


Fig 92

OBSERVATIONS ON VERY RARE TUMOURS OF THE OVARY

PRIMARY MESODERMAL MIXED TUMOURS OF THE OVARY

Fig. 93, 94 and 95 : Case 1.

Fig. 93 : Benign adenomyomatous part of the tumour. X 90.

Fig. 94 : Another part of the tumour showing the picture of carcino-sarcoma,
with islands of degenerate chondrous tissue. X 90.

Fig. 95 : Another part of the tumour showing the picture of endometrioid
adenocarcinoma. X 90.

In this case, the origin of the tumour from the associated endometriosis
was possible but not proved.

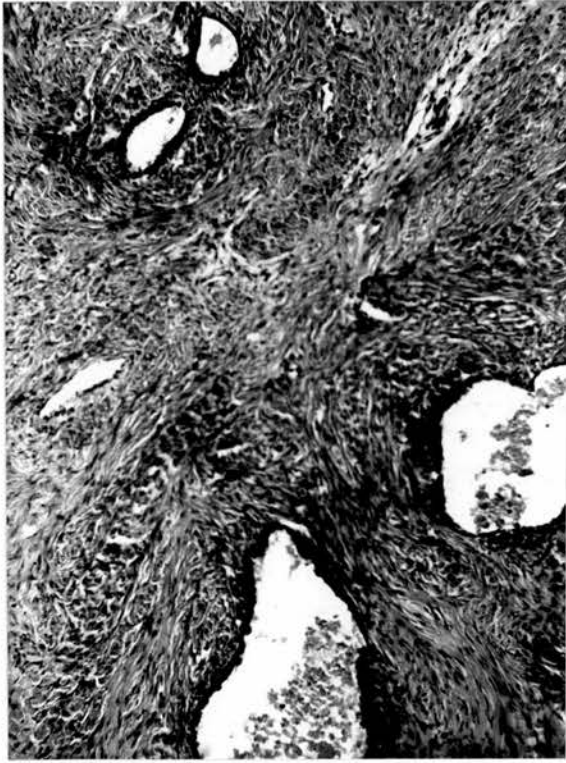


Fig. 93



Fig. 94

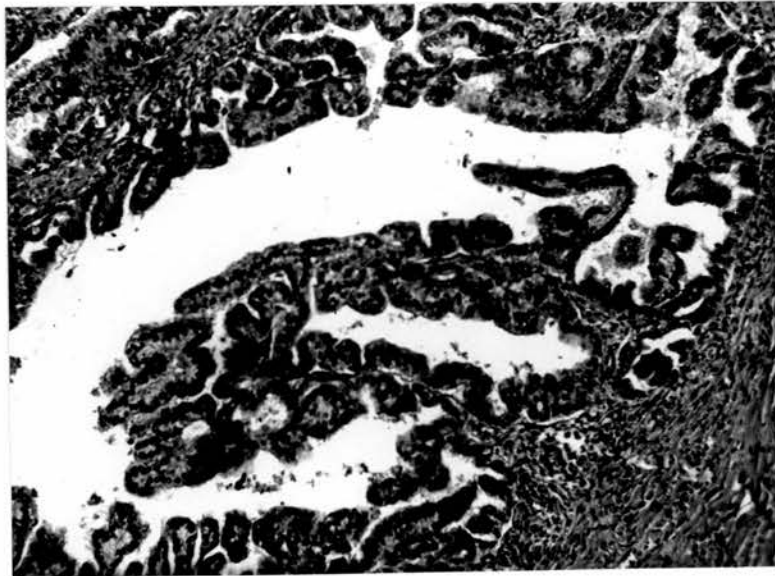


Fig. 95

Fig. 96, 97 and 98 : Primary mesodermal mixed tumour of the ovary. Case 2.

Fig. 96 : Epithelial elements in the tumour. X 100.

Fig. 97 : Rhabdomyoblasts. X 450.

Fig. 98 : Striated muscle fibres. Heidenhain's iron haematoxylin. X 1000.

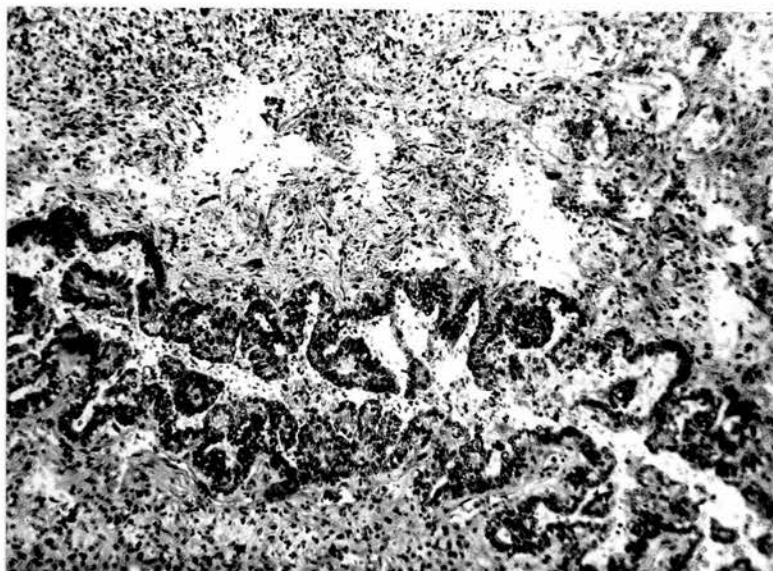


Fig. 96

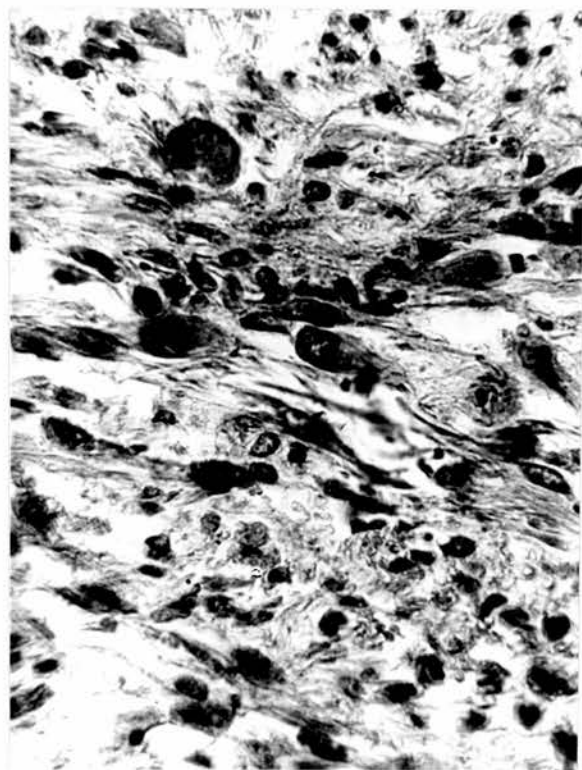


Fig. 97

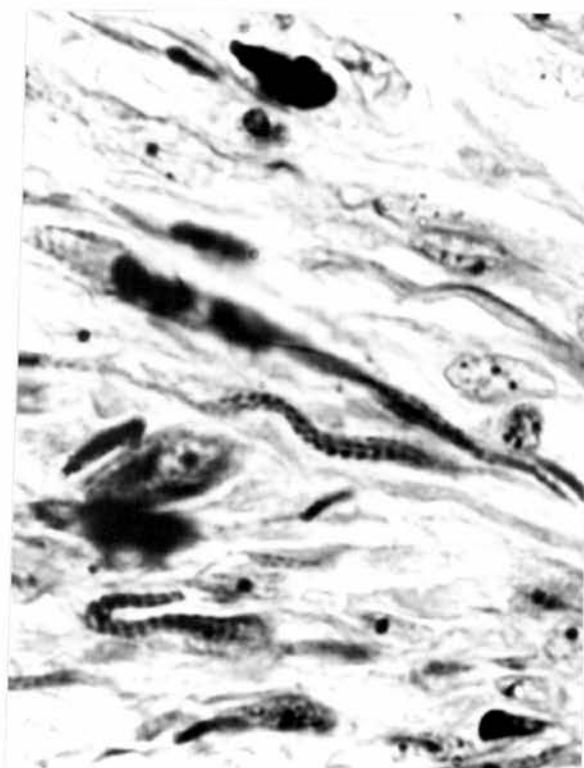


Fig. 98

MALIGNANT BRENNER TUMOUR

The diagnosis of malignant Brenner tumour should be based on strict criteria. Epithelial clumps in an ovarian carcinoma may simulate the appearance of the Brenner tumour, as is illustrated in this example.

Fig. 99 : Not a malignant Brenner tumour. Other parts of the tumour showed the picture of common ovarian carcinoma. There were no benign Brenner cell elements. X 140.

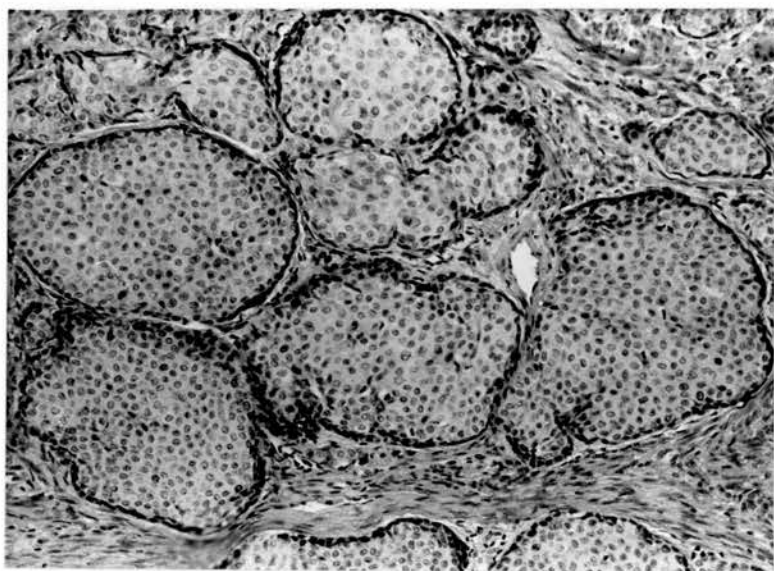


Fig. 99

Fig. 100, 101, 102 : Malignant Brenner tumour.

**Fig. 100 : Whole section showing the benign solid part (left)
and the malignant cystic part (right). X 10.**

Fig. 101 : Higher magnification of the benign solid part.

**The tumour looks inactive with central cystic
degeneration and peripheral hyaline degeneration.**

X 140.

Fig. 102 : Higher magnification of the malignant cystic part.

**The picture is that of a papillary cystadenocarcinoma,
with the multilayered malignant epithelium showing
a marked structural similarity to the Brenner pattern.**

Stromal invasion can also be seen. X 140.



Fig. 100



Fig. 101

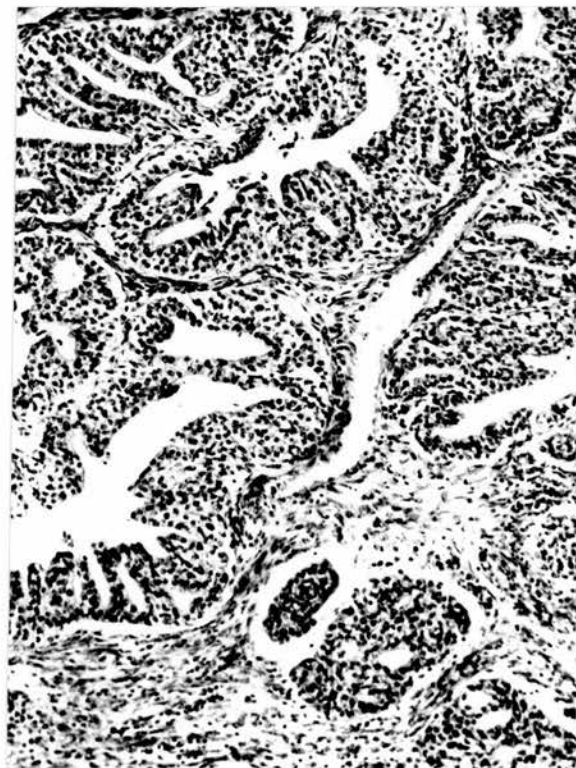


Fig. 102

MALIGNANT TERATOMA

Fig. 103 and 104 are from a case of solid well differentiated malignant teratoma. The histological picture of the primary tumour showed only adult mature tissues. The tumour, however, proved its malignant potential by recurrence after 6 months.

Fig. 103 : Brain tissue and chorioid plexus in the primary tumour.

Other mature tissues were also present. X 40.

Fig. 104 : Omental metastasis of neuroglial tissue. The peritoneal biopsies were composed only of this tissue, a feature known to occur with solid metastasizing teratomas.

X 40.

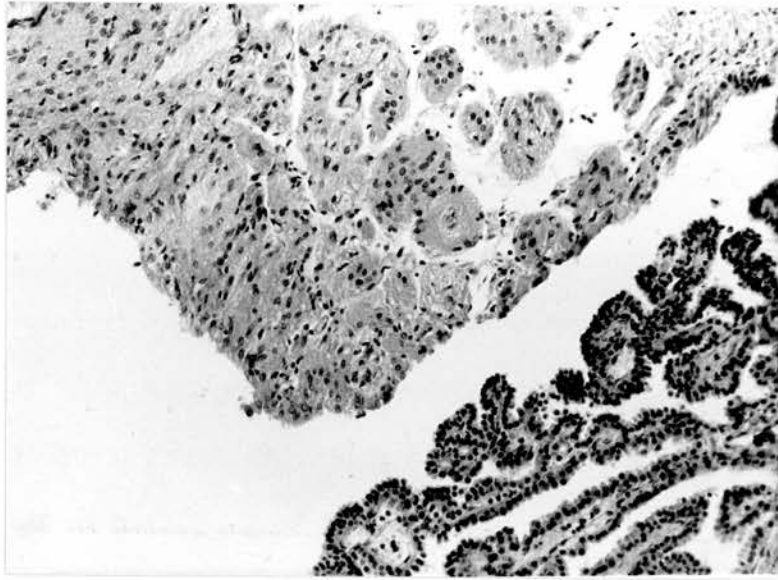


Fig. 103

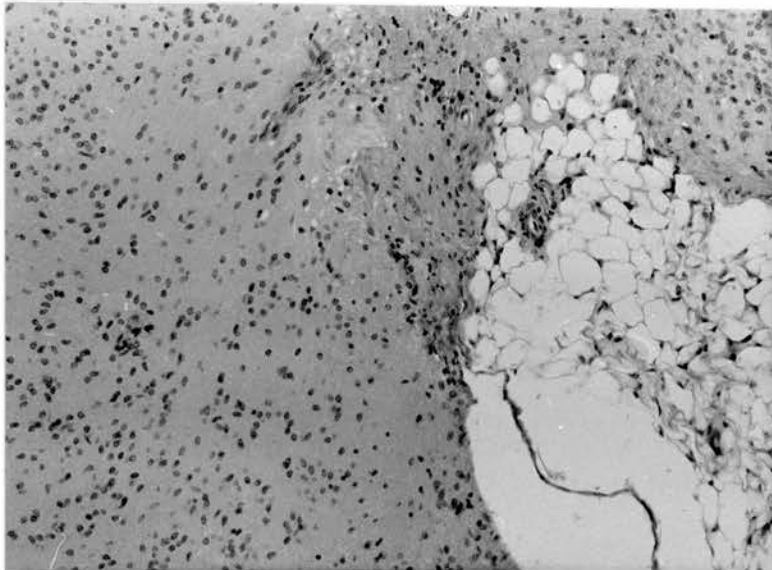


Fig. 104

MALIGNANT ADENOFIBROMA

Fig. 105, 106, 107, 108, 109, 110 : An unusual case of malignant transformation in an ovarian adenofibroma.

Fig. 105: The tumour was partly solid and partly cystic.

This figure shows the picture of the cystic part. A lining of benign cubical epithelium with occasional stunted papillae and psammoma bodies can be seen. X 20.

Fig. 106: Solid part of the tumour. Small glands widely dispersed in fibrous tissue, typical of benign adenofibroma, can be seen. X 40.

Fig. 107: Solid part of the tumour. A gradual transition into an adenomatous pattern can be seen. X 25.

Fig. 108: Solid part of the tumour. Glands are crowded together in compact clusters. X 40.

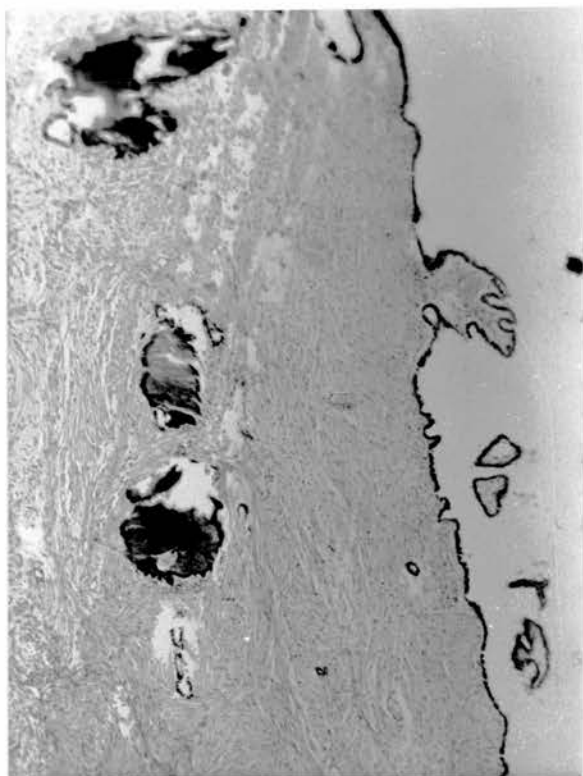


Fig. 105

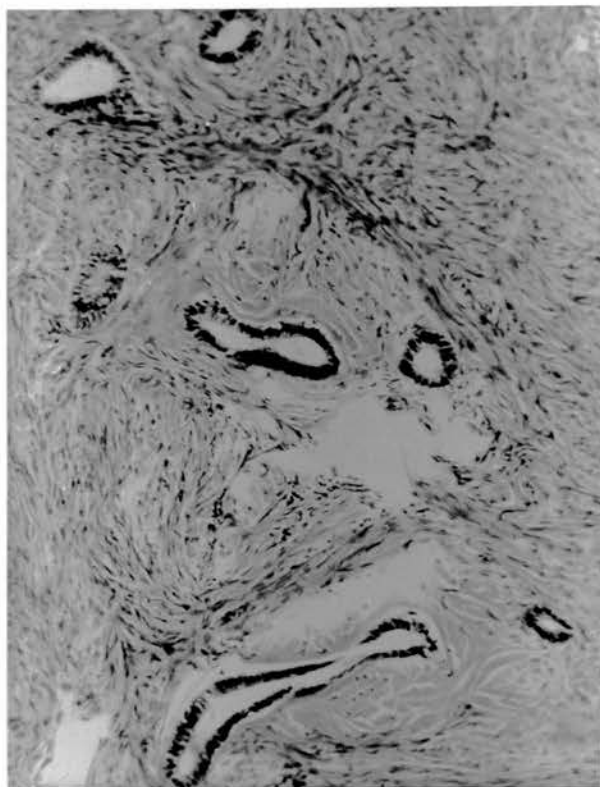


Fig. 106

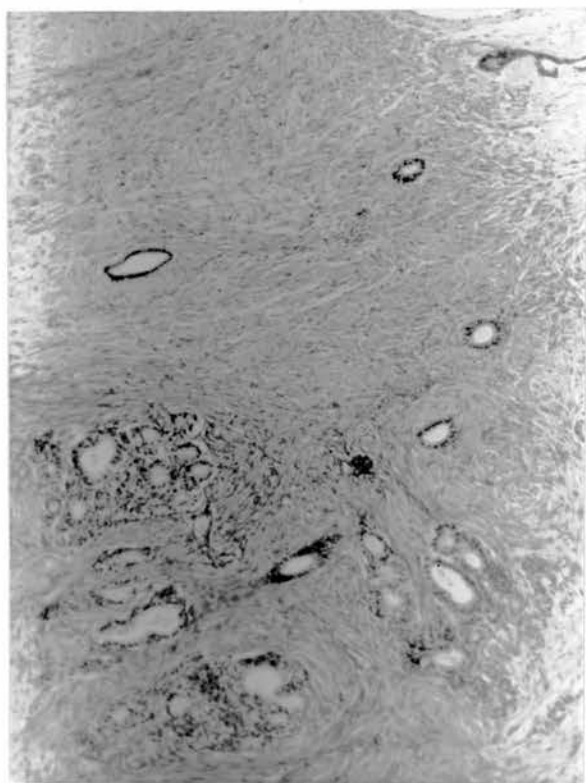


Fig. 107

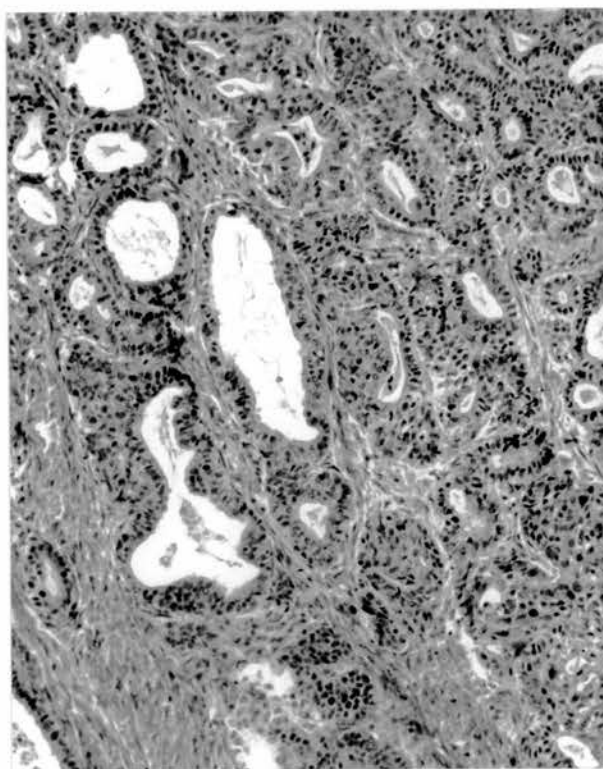


Fig. 108

Fig. 109 : Solid part of the tumour (see previous 3 figures).

The gland pattern is lost , the cells are arranged in clumps and columns and the picture is that of frank malignancy. X 40.

Fig. 110 : Solid part of the tumour. The picture here is that of an anaplastic growth. A prominent peri-epithelial thecal reaction is present in the stroma(The postmenopausal endometrium showed a moderate degree of proliferative activity). X 63.

Fig. 106 - 110 are from one histological section and leave no doubt about the transition from one picture to the other.

Fig. 111 and 112 : Another example of malignant transformation in an adenofibroma.

Fig. 111 : Benign part of the tumour. X 25.

Fig. 112 : Malignant part of the tumour. X 40.

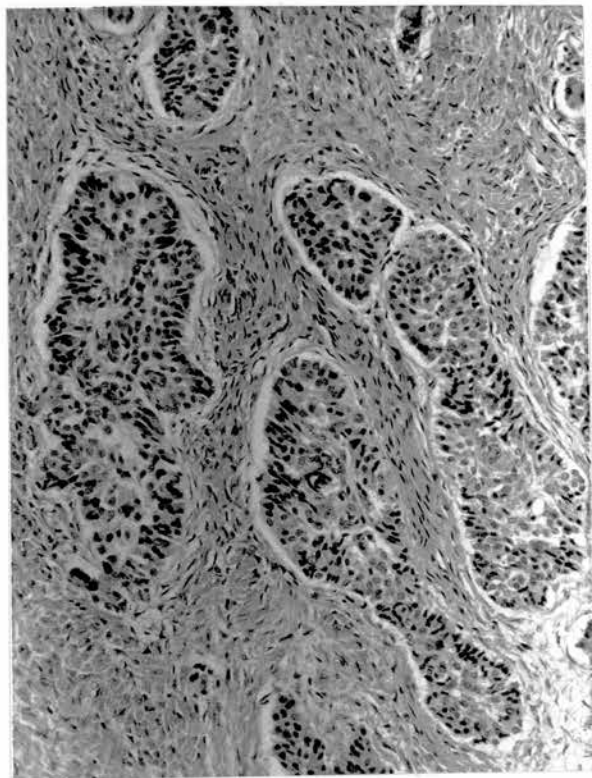


Fig.109

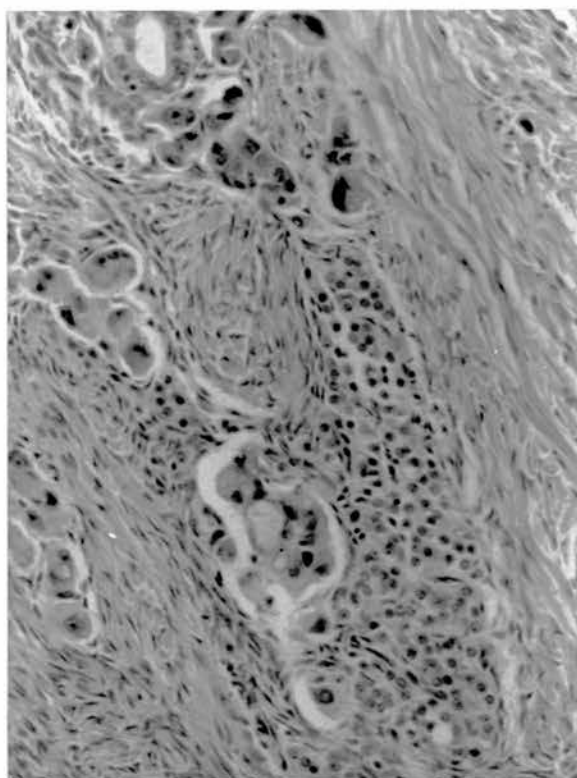


Fig.110

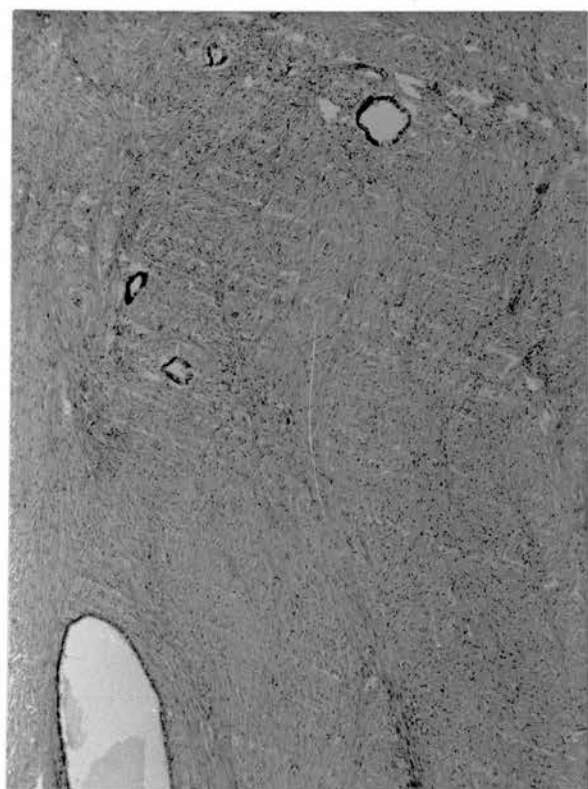


Fig. 111

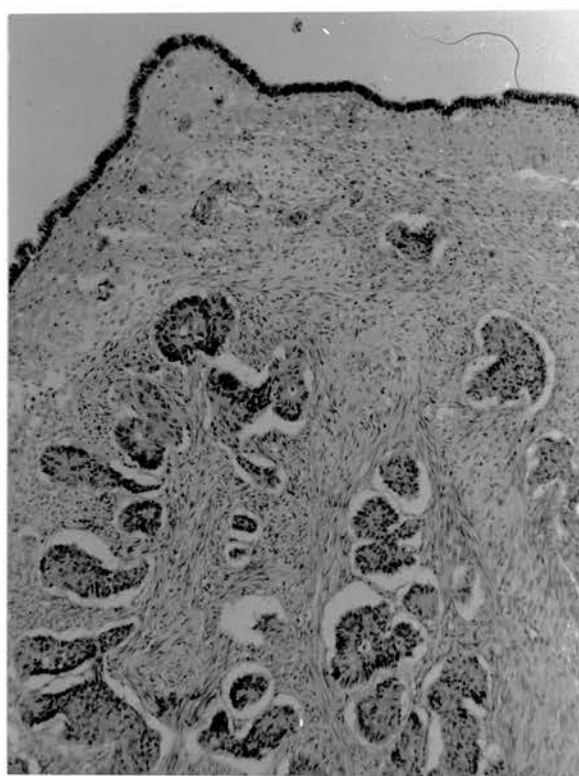


Fig.112

MESOBLASTOMA OF TEILUM

A considerable amount of confusion exists in the literature about the status of this tumour and especially its relationship to the tumour described as mesonephroma by Schiller and the related clear cell carcinoma. It seems that this is partly due to different authors speaking about different tumour types , and not the same type of tumour.

Fig. 113 : A typical example of the clear cell carcinoma. X 63.

Fig. 114 : The picture described as mesonephroma. Note the hob-nail epithelium lining the epithelial spaces (right) and the clear cell pattern (left).

The tumour is essentially an epithelial tumour. X 63.

Fig. 115 : Mesoblastoma. The main characteristic of the tumour , as described by Teilum, are the primitive mesenchymal type of stroma and the paucity of epithelial elements. Teilum interprets the epithelial spaces as representing yolk sac endoderm (Mesoblastoma vitellinum). X 63.

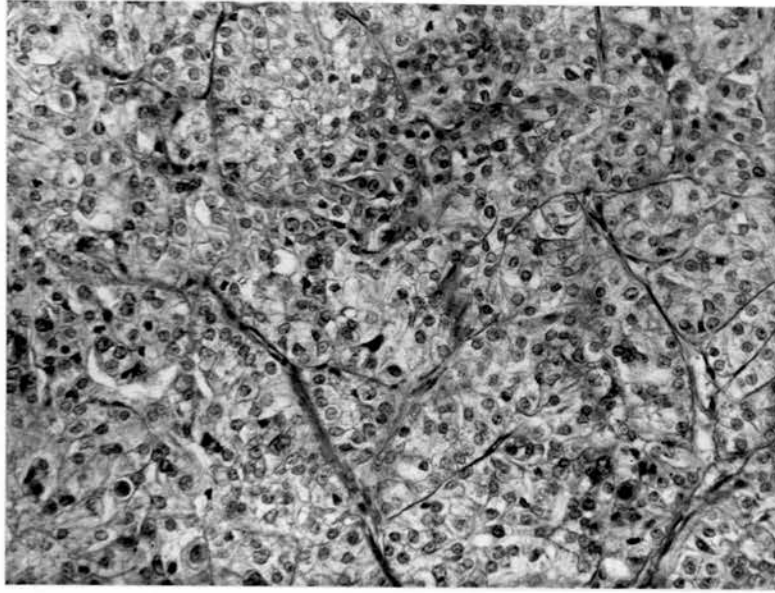


Fig. 113

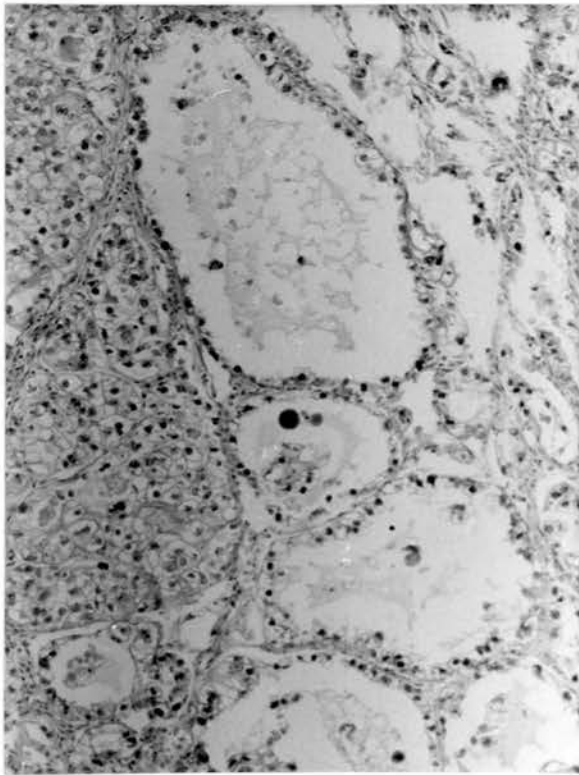


Fig. 114

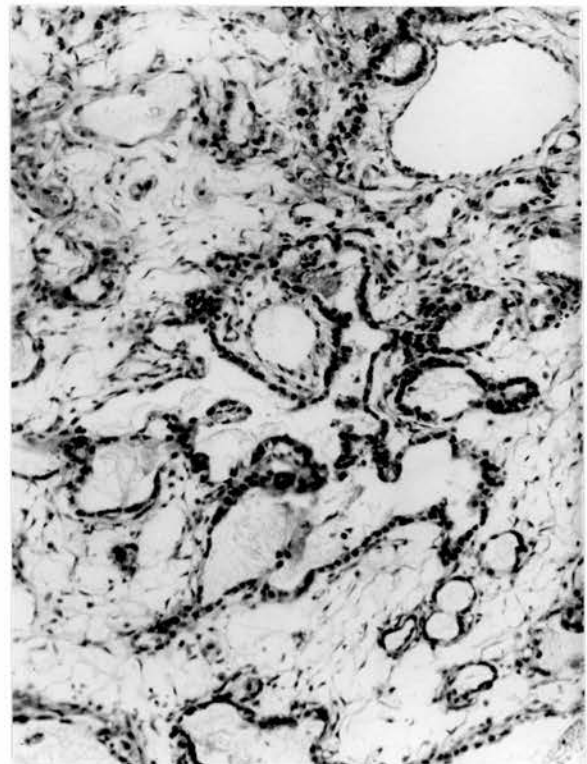


Fig. 115

OVARIAN ENLARGEMENT SIMULATING TUMOUR FORMATION
IN A VIRILIZED PATIENT DURING PREGNANCY

Fig. 116 : Ovary removed at Caesarean section. External surface.

Fig. 117 : Ovary removed at Caesarean section. Cut surface.

Multiple small peripheral cysts can be seen. The ovarian enlargement, however, is mainly solid.

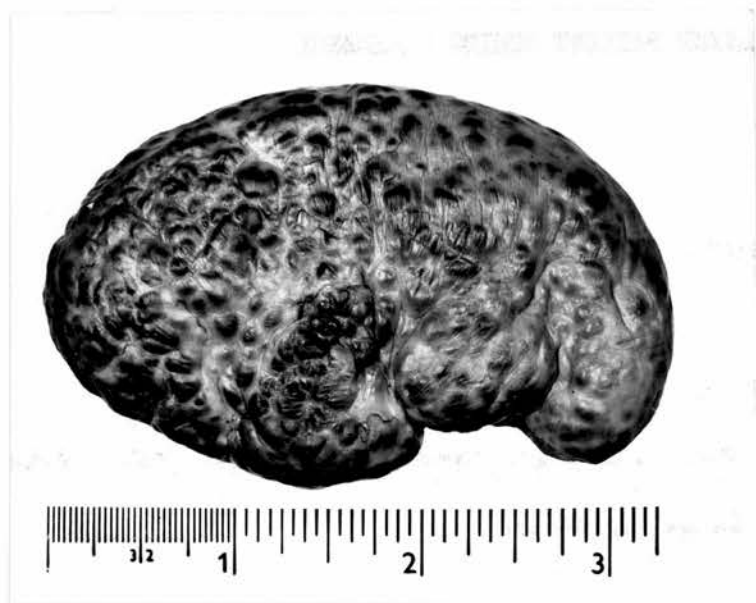


Fig. 116

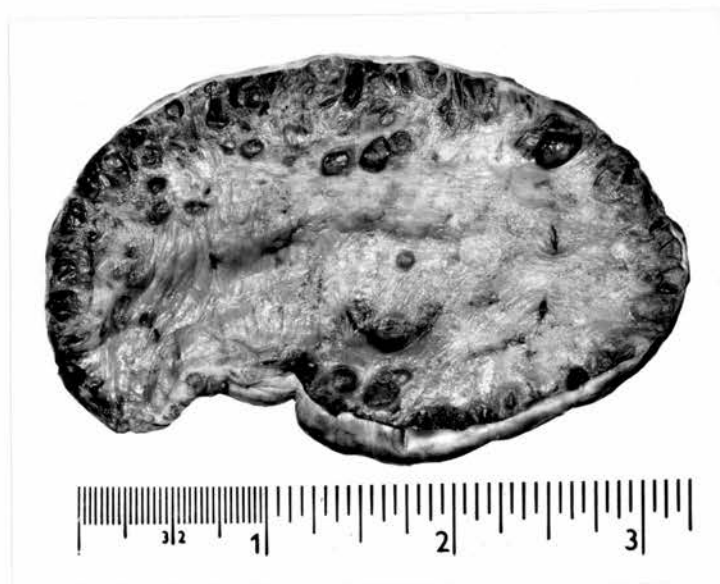


Fig. 117

Fig. 118 : Resected wedge from the other ovary at second Caesarean section.

Solid nodules of variable sizes can be seen in the cystic ovary.

The microscopical picture of the cysts and the solid nodules is shown below.

Fig. 119 : Microscopical appearance of the cysts shown in the previous figure.

Multiple layers of luteinized theca cells are seen surrounding the cystic follicles. The granulosa cell layer is atrophic. X 100.

Fig. 120 : Microscopical appearance of the solid nodules, shown in the resected wedge above. The nodules are made up of luteinized theca cells.

X 250.



Fig.118

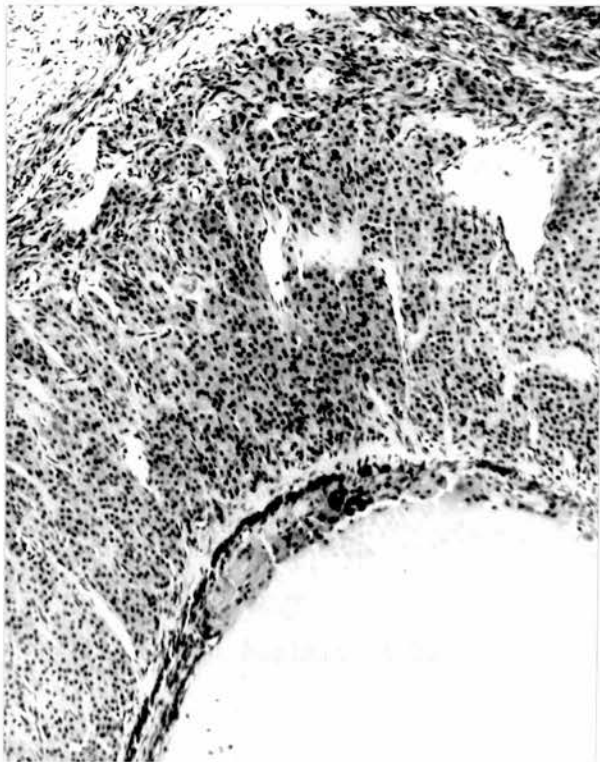


Fig.119

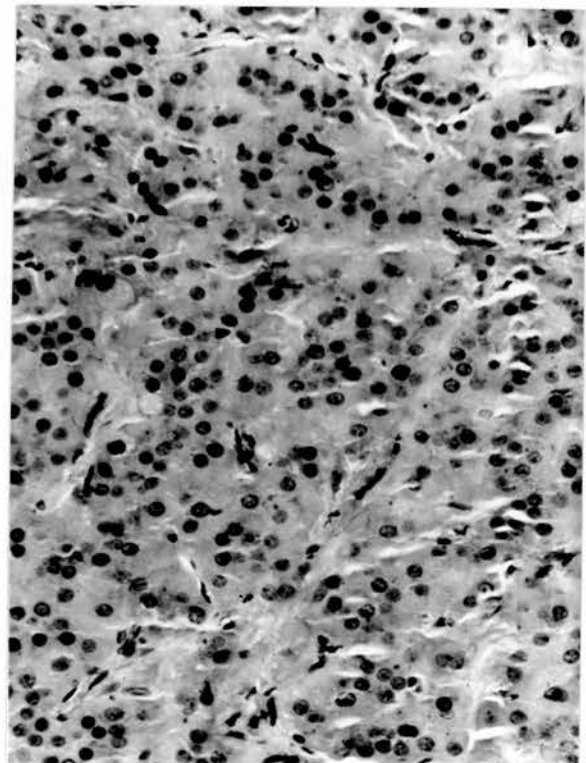


Fig. 120

Fig. 121 : A small focus of theca lutein cells in the hyperplastic ovarian stroma.

Several such foci were scattered in the ovary. X 250.

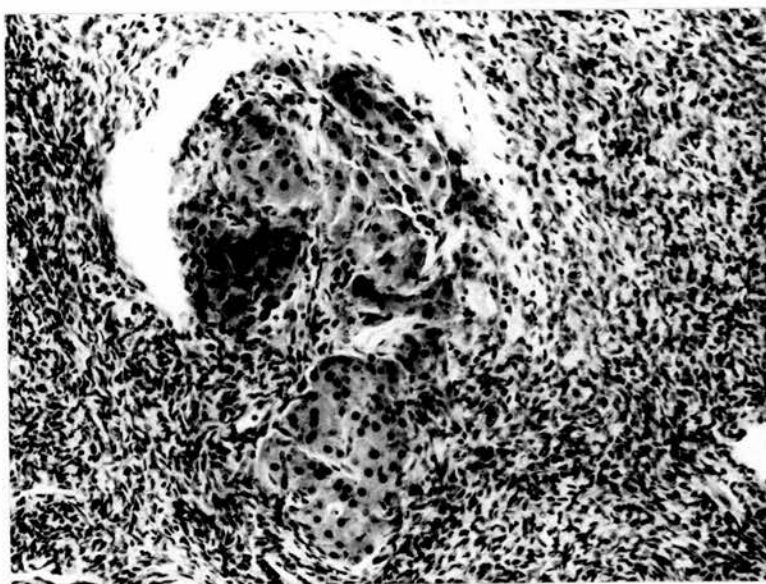


Fig. 121

SEX CHROMATIN AND CHROMOSOME ANALYSIS IN OVARIAN TERATOMAS

Fig. 122 : Sex chromatin in the stratified squamous epithelium of teratoma.

X 1000.

Fig. 123 : Sex chromatin in respiratory epithelium in teratoma.

X 1000.

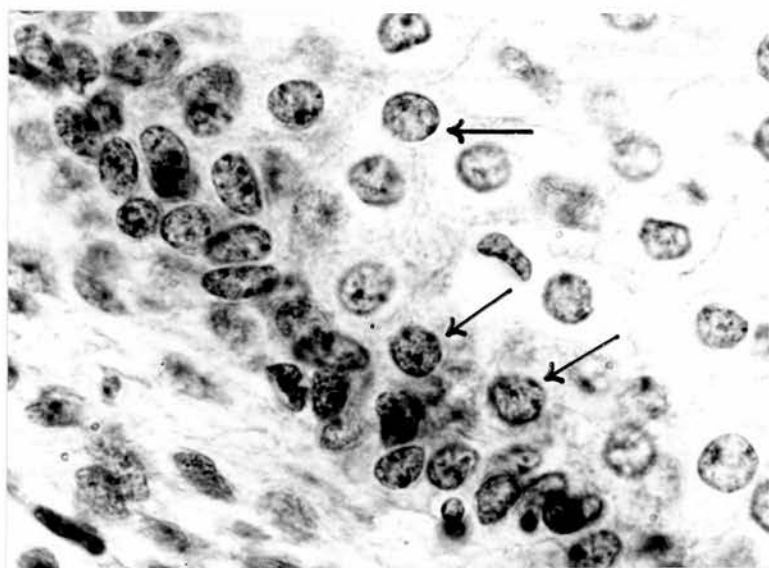


Fig. 122

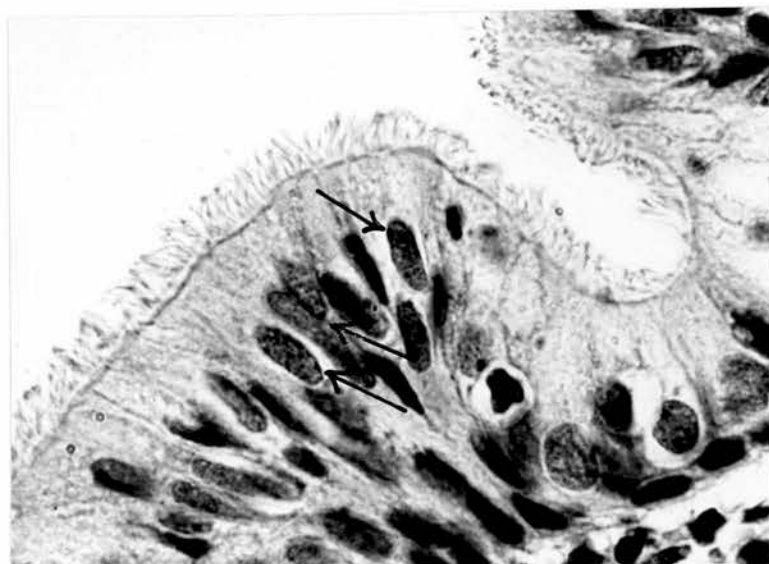


Fig. 123

Fig. 124 : Sex chromatin in neuroglial tissue in teratoma.

X 1000.

Fig. 125 : Sex chromatin in cartilage in teratoma.

X 1000.

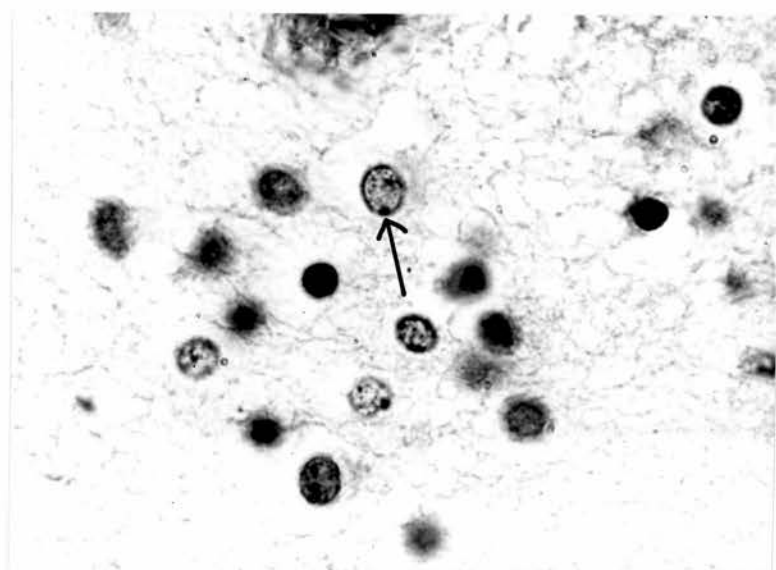


Fig. 124

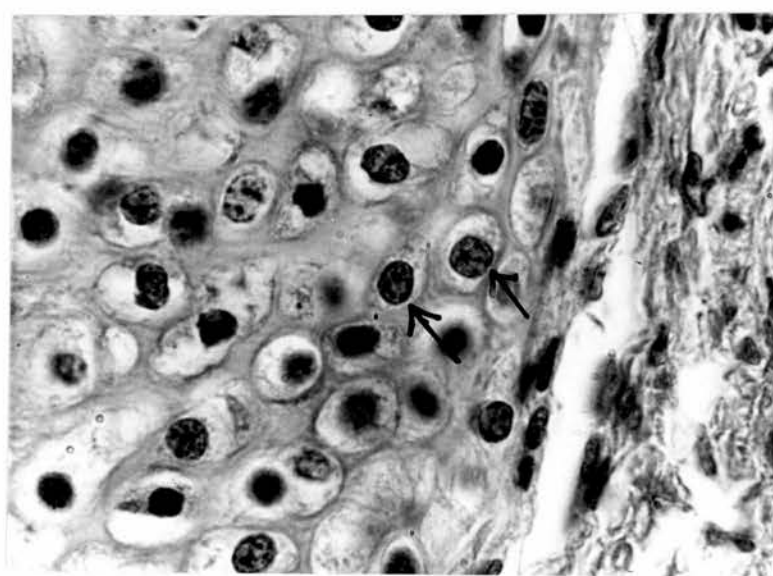


Fig. 125

Fig. 126 : Karyotypes in six benign cystic teratomas.

A normal 46/XX pattern .



Fig. 126

THE RELATIONSHIP BETWEEN OVARIAN TUMOURS AND INTERSEX STATES WITH SPECIAL
REFERENCE TO THE DISGERMINOMA AND ARRHENOBLASTOMA

Fig. 127 : Case 2. Ovarian disgerminoma.

Corpus luteum in close proximity to tumour cells. X 100.

Fig. 128 : Case 2. Ovarian disgerminoma.

Sex chromatin in the fibroblasts of the tumour stroma.

The tumour nuclei are not suitable for analysis. X 1000.

Fig. 129 : Case 2. Ovarian disgerminoma.

Chromosome analysis of peripheral blood leucocytes.

A normal 46/XX karyotype.

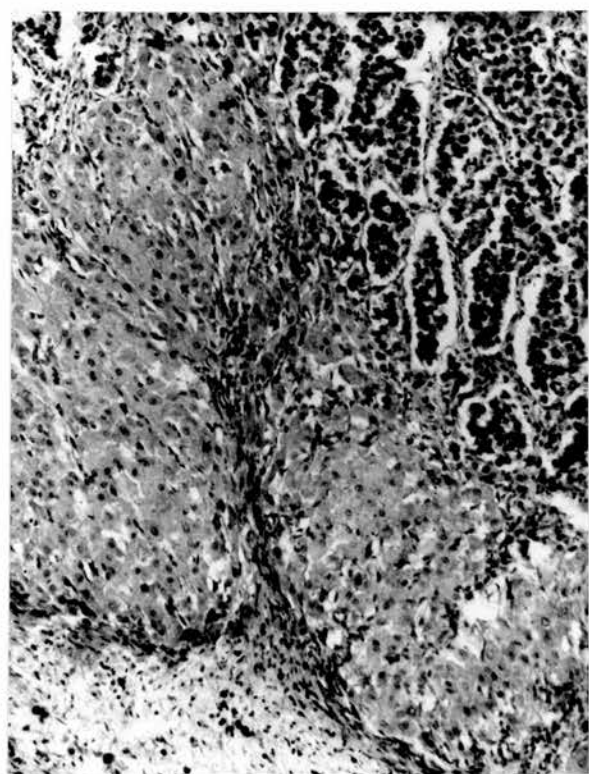


Fig. 127

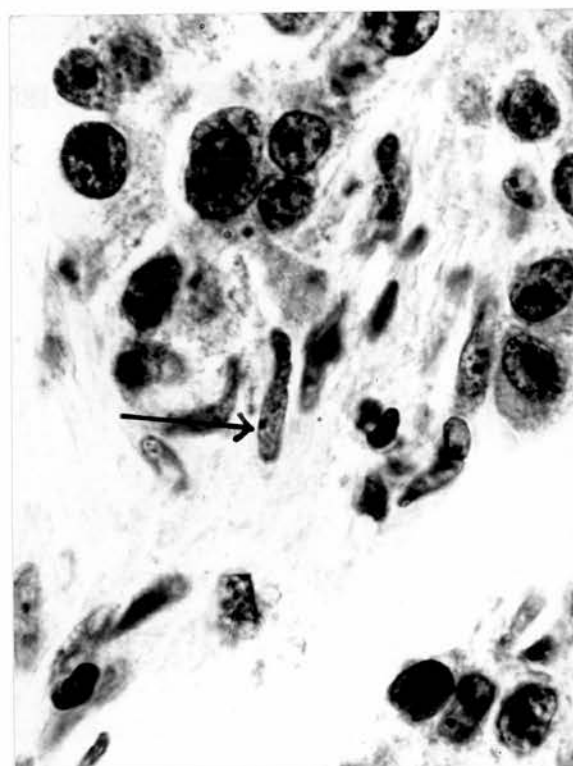


Fig. 128

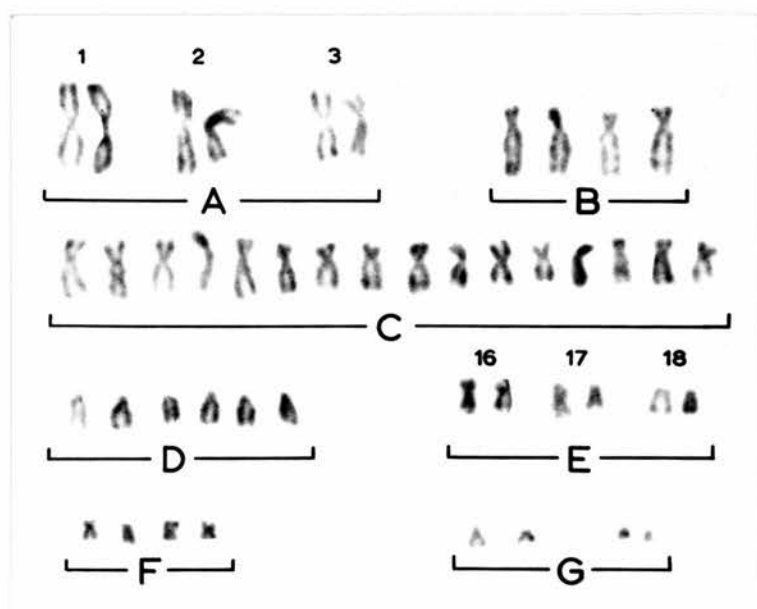


Fig. 129

Fig. 130 : Case 3. Disgerminoma.

Microscopical picture of the tumour. X 175.

Fig. 131 : Case 3. Disgerminoma.

Sex chromatin in the fibroblasts of the tumour stroma. X 1000.

Fig. 132 : Case 3. Disgerminoma.

Chromosome analysis of peripheral blood leucocytes.

A normal 46/XX karyotype.

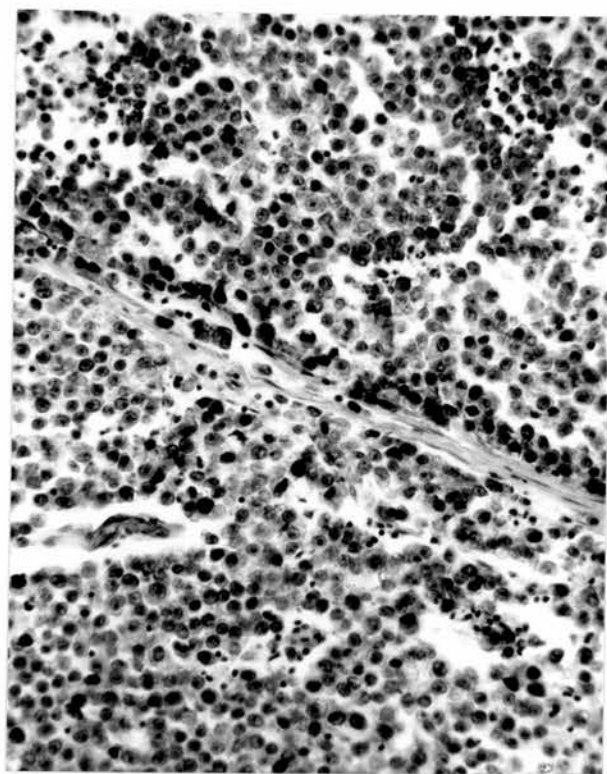


Fig. 130

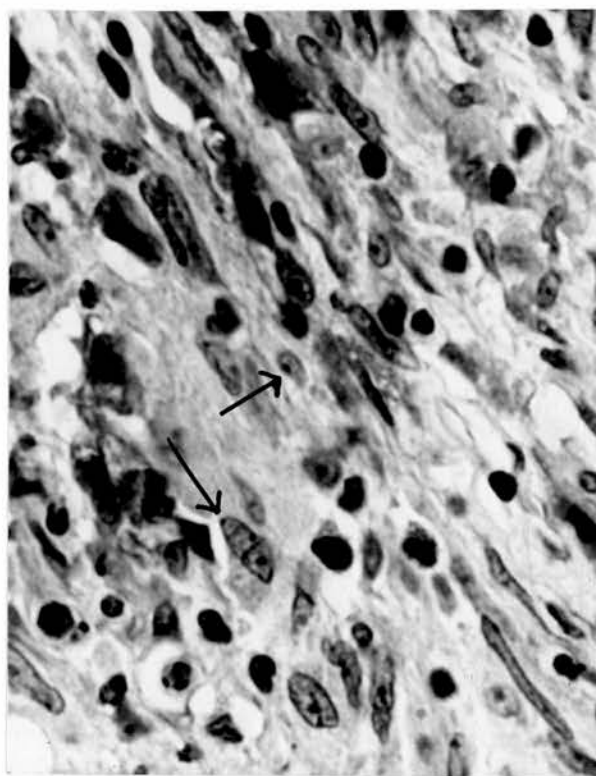


Fig. 131

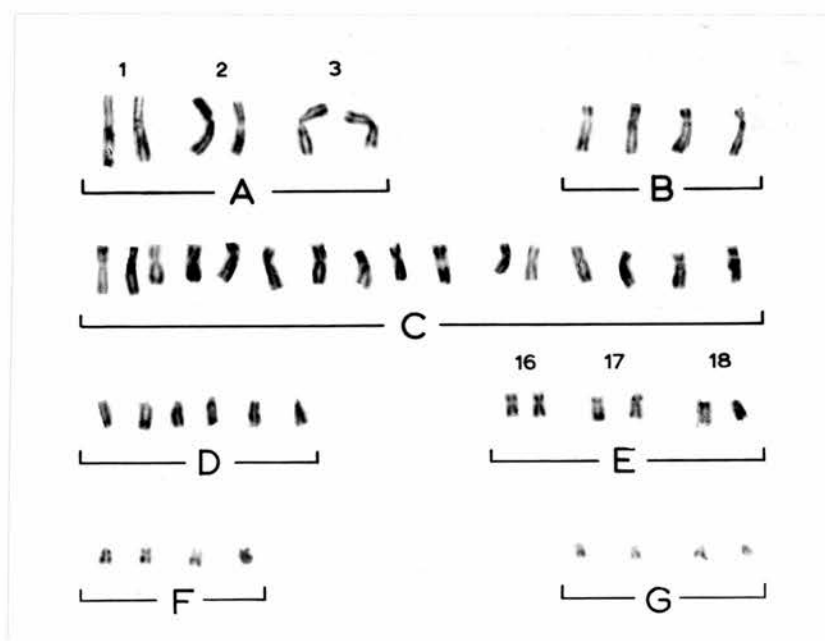


Fig. 132

Fig. 133 : Case 4. Disgerminoma.

Microscopical picture of the tumour.

A lymphocytic collection is seen. X 175.

Fig. 134 : Case 4. Disgerminoma.

Chromosome analysis of peripheral blood leucocytes.

A normal 46/XX karyotype.

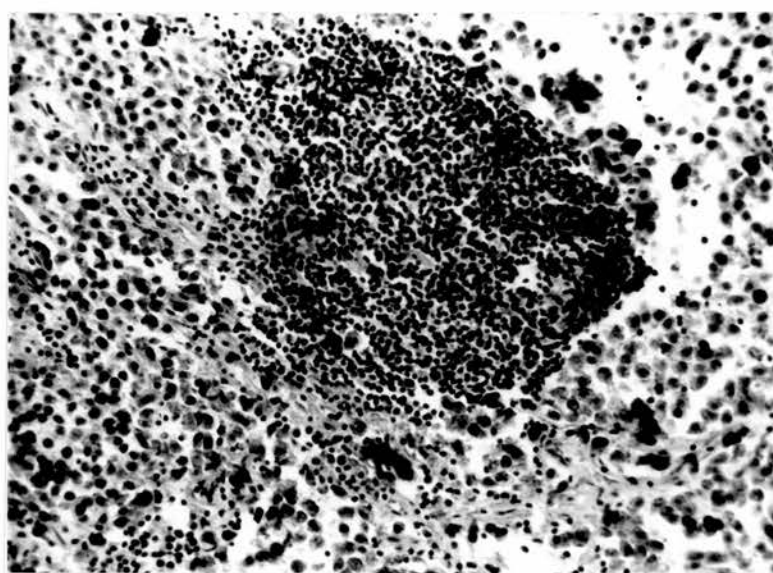


Fig. 133

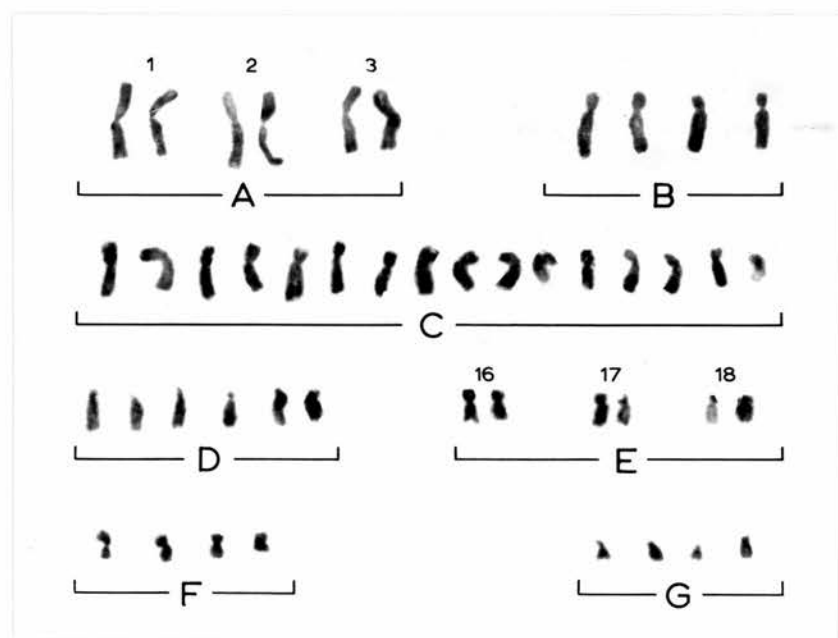


Fig. 134

Fig. 135 : Case 6. Testicular tubular adenoma.

The case proved to be one of testicular feminization.

X 40.

Fig. 136 : Case 8. Arrhenoblastoma.

Chromatin positive cells. X 1000

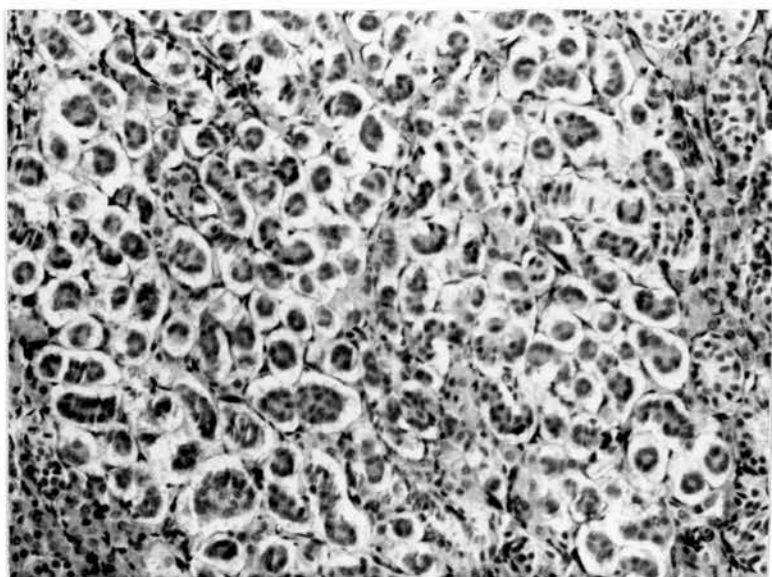


Fig. 135

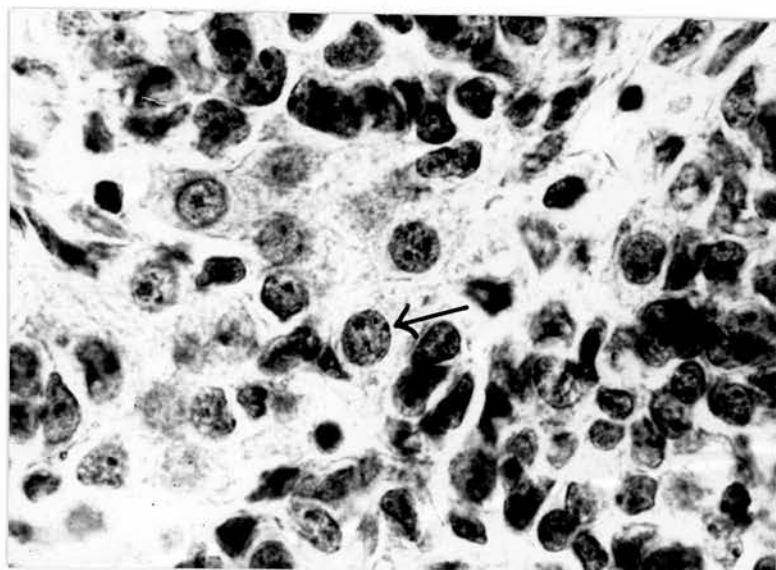


Fig. 136

THE OCCURRENCE OF GRANULOSA AND
THECA TUMOURS IN CLINICALLY
NORMAL OVARIES

BY

M. F. FATHALLA

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THE OCCURRENCE OF GRANULOSA AND THECA TUMOURS IN CLINICALLY NORMAL OVARIES

A study of 25 cases

BY

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Department of Obstetrics and Gynaecology, University of Edinburgh

THE aim of this report is to draw attention to a special group of granulosa and theca cell tumours which cause no significant enlargement of the ovary and betray their presence only by evidence of abnormal oestrogenic activity.

Of 91 cases of granulosa and theca cell tumours, examined in the pathology laboratory of the Department of Obstetrics and Gynaecology of the University of Edinburgh since 1950, 25 tumours were encountered in ovaries that were normal-sized or only slightly enlarged.

CLINICAL FEATURES

The age of the patients in the 25 cases ranged from 47 to 80 years with only one patient below the age of 50 years. In our total series of 91 granulosa and theca cell tumours of all sizes, ages ranged between 17 and 86 years with 23 patients below the age of 50 years. Eleven patients with the small tumours were nulliparous and all were postmenopausal for periods varying from 2 to 31 years.

With the exception of three cases, the presenting symptom was uterine bleeding. Of these three patients, one was operated upon for large uterine fibroids, another had a large twisted fibroma of the opposite ovary and the third had an adenocarcinoma of the opposite ovary. The duration of the history of postmenopausal bleeding varied from 2 weeks up to 6 years. Delays were largely due to reluctance on the part of the gynaecologist to operate in the absence of a palpable tumour. Thus, only 11 of the patients had a laparotomy within one year of the start of the bleeding, 6 patients were operated upon in the second year, 3 in the third year, one after 4 years and one after 6 years.

The management of the patients presenting with postmenopausal bleeding followed several lines. In two patients laparotomy was the first line of treatment. In 12 patients laparotomy was performed after an initial curettage showing endometrial hyperplasia (7 cases) or carcinoma (5 cases). In two patients curettage was performed and hysterectomy was done when the bleeding recurred. In four patients the decision for laparotomy was only taken after curettage had been performed two or three times. In two patients, after an initial curettage, deep X-ray therapy to stop ovarian function was given. In both cases, the treatment failed. One of these two patients had the X-ray treatment repeated three times and was only operated upon after three years when an early adenocarcinoma of the endometrium was also discovered. The second of these two patients had a laparotomy after intermittent bleeding for six years.

It is interesting to note that a history of radiation menopause was present in three other cases in this series. One patient had a radium-induced menopause for the treatment of dysfunctional premenopausal bleeding 18 years before. Another patient had the same treatment 31 years before the occurrence of postmenopausal bleeding and the detection of an endometrial adenocarcinoma. The third patient received radiotherapy for the treatment of fibrosarcoma of the groin at the age of 28 years. Her periods had stopped since then and she presented 24 years later with uterine bleeding and endometrial hyperplasia.

Oestrogen assays were available in two of the patients with postmenopausal endometrial hyperplasia. In one patient, aged 75 years and postmenopausal for 27 years, the 24 hours urine

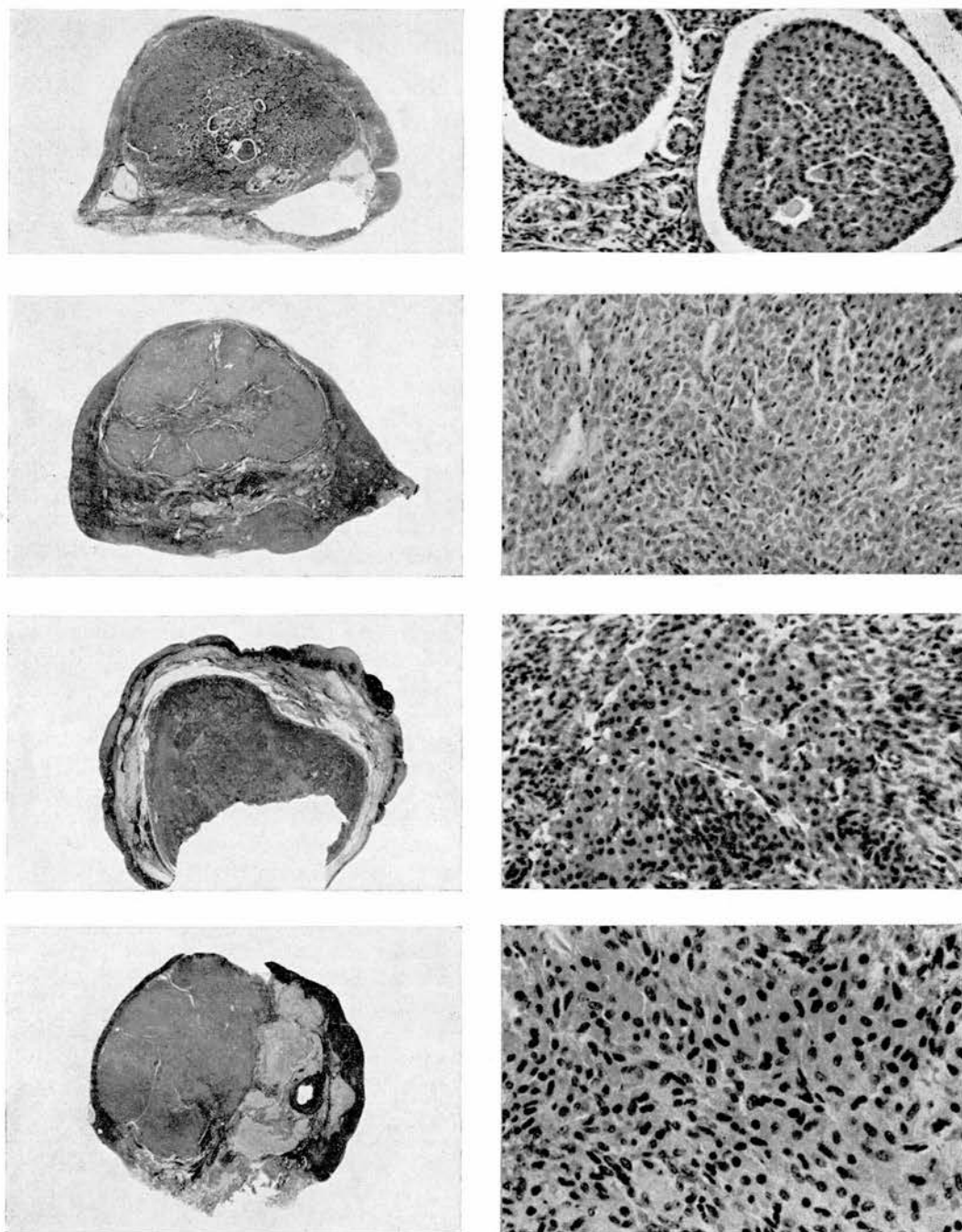


FIG. 1

Examples of granulosa and theca cell tumours in clinically normal ovaries. Figures on the left side show a complete section of the ovary ($\times 3$). On the right side, the microscopical appearance of each tumour is shown ($\times 40-63$). The appearance of luteinization is noticeable in the last two tumours.

volume contained a total of $17.8 \mu\text{g.}$ oestrogens. In the second patient, 66 years old and 10 years postmenopausal, the total oestrogens were $38.7 \mu\text{g.}$ per 24 hours urine volume. The total mean urinary oestrogen excretion in the normal postmenopausal patient is of the order of $5.8 \mu\text{g.}$ per 24 hours urine with a range of $3.1 \mu\text{g.}$ to $8.1 \mu\text{g.}$ (Kellar *et al.*, 1959).

PATHOLOGICAL FEATURES

All the tumours were unilateral, with the exception of one case of bilateral thecoma. Seventeen of the tumours were thecomas and 8 were granulosa cell tumours (Fig. 1). (In our total series of 91 cases, only 28 were thecomas.) The appearance described as luteinization was more frequently observed and also more marked than in the large tumours. In three of the cases, the tumour was almost completely luteinized. In spite of their small size the tumours often showed evidence of old fibrosis; hyalinization was usually extensive and in some cases it replaced a large part of the tumour. The ovary, apart from the tumour, was commonly the seat of a variable degree of cortical stromal hyperplasia. In one case foci of lutein cells were included in the hyperplastic ovarian stroma. Hyperplasia of the hilus cells was prominent in one case. A small fibroma was also present in the same ovary in one case.

In three patients the endometrium was inactive. These were the three patients already mentioned, who presented with other large tumours in the uterus or opposite ovary. In 15 cases the endometrium showed marked proliferative activity or cystic hyperplasia. In the remaining 7 cases endometrial carcinoma was present. In 5 out of these 7 cases, the ovarian tumour was a thecoma. It is also worth noting that in the total series of 91 cases of granulosa and theca cell tumours, 11 cases of concomitant endometrial carcinoma were encountered. In other words, in 7 out of 11 cases in which a granulosa or theca cell tumour was associated with endometrial carcinoma, the ovary showed no clinical enlargement. Two of the cases of endometrial carcinoma in this series were of special interest. Endometrial curettage, two years previously in one patient and three years previously in the other patient, showed postmenopausal active hyperplasia. The second patient received in the interval deep X-ray therapy to stop ovarian function.

DISCUSSION

Although granulosa and theca cell tumours of very small size have been noted occasionally in previously published series (Biggart and Macafee, 1955), their frequency and significance are not sufficiently realized. In our material 25 out of 91 granulosa and theca cell tumours were found in ovaries which showed no clinical enlargement. The real incidence of these small tumours may even be higher since they are more liable to be missed than the larger tumours, especially if they are hormonally inactive. The relative frequency of these tumours should direct attention to their possible presence in the postmenopausal patient presenting with evidence of abnormal oestrogenic activity, even when the ovaries are palpably normal.

Several observations suggest that these cases do not simply represent early tumours but rather a variant of granulosa and theca cell tumours characterized by a very slow and possibly limited capacity for growth. Thus, the tumours were encountered at a later age than the larger tumours. Furthermore, the history of these patients often revealed evidence of long standing abnormal oestrogenic activity before operation. Pathological examination also often showed a pronounced degree of fibrosis and hyalinization in the tumours. In the present state of knowledge factors governing the rate and capacity of growth in tumours are not well understood. However, two pathological features in our series are worthy of note in this connexion. The first is the predominance of the thecal type of tumour and this is in accord with the observation that thecomas are generally more slowly growing and relatively more benign than the granulosal type of tumour. The second feature is the more frequent and more marked appearance of luteinization in these small tumours. Since this distinctive histological appearance is probably indicative of steroid storage in the cells, it may be suggested that there is a correlation between a low potential for growth and a capacity of the cells for steroid storage. It may also be noted that the so-called lipoid cell tumours of the ovary are usually of a small size (Haines and Taylor, 1962).

The association of endometrial carcinoma with feminizing mesenchymal tumours has been reported, the figures varying from 3 to 27 per cent (Hertig and Gore, 1963). In our total series of 91

granulosa and theca cell tumours, this association was noted in 11 cases. Of these, 7 were encountered among the 25 cases of small tumours and only 4 were encountered in the remaining 66 cases of larger tumours. This relatively high frequency of associated endometrial carcinoma with the small tumours demands an explanation, and several may be suggested. One may be the predominance of thecomas in this series, since thecomas more frequently have this association than granulosa cell tumours (Greene, 1957). The greater age of the patients may be another factor. It is also possible that more of these small tumours are detected when the endometrium is malignant than when it is benign, because operation is less readily undertaken when the endometrial curettings are benign. However, despite all these possible factors, the frequency of the association is not fully explained. In two cases in this series the progression from postmenopausal hyperplasia of the endometrium to adenocarcinoma was noted after two years in one patient and three years in the other. In all probability the small tumour was present over these periods. The possibility of the presence of a small undetected functioning tumour of the ovary in a case of endometrial carcinoma should be remembered. Such a tumour may be responsible for failure of treatment by radium, as in a case mentioned by Sparling (1950).

The possibility that radiation may play a role in the development of feminizing ovarian tumours in the human as well as in the experimental animal has been suggested (McKay *et al.*, 1953). Five patients in this series received radiotherapy, usually to stop ovarian function, before the discovery of the tumour. It is possible, however, that some of the patients may have had the tumour at the time of the radiotherapy. Indeed, this appears to be the most plausible explanation in two of our cases as well as in some other reported cases (Maxwell, 1957; Sparling, 1950; Pratt, 1950). It is important to remember that a patient bleeding around the time of the menopause and showing evidence of abnormal oestrogenic activity may have an undetected functioning ovarian tumour, especially if a

standard dose of radiotherapy fails to stop ovarian function. In two other cases in this series the long interval of amenorrhoea between the induction of menopause for abnormal uterine bleeding and the presentation of the tumour makes it more probable that the tumour developed after the menopause. In these two cases, however, it may be argued that the aetiological relation, if any, is with the ovarian dysfunction rather than with the irradiation.

SUMMARY

The frequency with which granulosa and more particularly theca cell tumours may be present for long periods without causing clinical enlargement of the ovary is not generally realized. Twenty-five out of 91 tumours were found to be of this type. These small tumours have a very slow and possibly limited capacity for growth but are endocrinologically active. Endometrial carcinoma was present in 7 out of 25 cases. A history of ovarian irradiation was present in 5 cases.

ACKNOWLEDGEMENT

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SEX CHROMATIN AND
CHROMOSOME ANALYSIS IN
OVARIAN TERATOMAS

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Sex chromatin and chromosome analysis in ovarian teratomas

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THE DEVELOPMENT of ideas about the nature and origin of teratomas provides an interesting reflection of the rate of progress in methods of scientific investigation. Theories of teratogenesis may be seen to have followed four phases.

The striking fetuslike characteristics of the teratoma have been known for centuries and these encouraged the belief that they were the product of conception in the form of an included twin or an ovarian pregnancy or as the outcome of parthenogenesis.¹ These speculations were based on simple observations of the gross morphology of teratomas.

Later, the application of rigorous histopathologic techniques to these specimens quickly exposed the inaccuracies and exag-

gerations of earlier descriptions and discredited the idea of the fetal nature of these tumors.² The alternative suggestion was developed that teratomas are neoplasms originating from foci of cells which, at a very early stage of embryogenesis, escape from the control of primary organizers.³ These cells retain their primitive totipotency and may produce tumors which contain tissues representative of all three germ layers, but they totally lack any vertebral organization.

The recent demonstration of sex chromatin⁴ provided one method of testing these conflicting hypotheses. The concept of the teratoma as a neoplasm implied that it should have the same genetic sex as the host, whereas older ideas of the fetal nature of the teratoma suggested that there might be some discrepancy between the sex of the host and the tumor. The first report of sex chromatin studies on teratomas revealed such

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a disparity in that some testicular teratomas proved to be chromatin positive.⁵ Since then a large number of similar studies on both female⁶⁻²⁰ and male²¹ teratomas have been reported. Undoubtedly, sex chromatin gives invaluable information about the X chromosome complement of the cell. However, it is less reliable in malignant tissues²²; it is subject to technical misinterpretations²²; it gives no idea of the Y chromosome complement of the cell; and it gives no information about autosomes.

Consequently, it is now necessary to supplement sex chromatin studies with full chromosome analysis. So far, only three reports describing a total of 8 teratomas have been described.²³⁻²⁵ This communication describes a further study of sex chromatin in 187 benign ovarian teratomas, and the chromosome analysis of 6 of these specimens.

Material and methods

The material for the sex chromatin study was selected from the material collected in this department over the past 10 years. One hundred and eighty-seven specimens were considered adequate for study. All of these were benign ovarian cystic teratomas with the single exception of one benign solid teratoma. Each specimen was fixed in formalin and slides were stained with hematoxylin and eosin. In a few cases, additional sections were cut and stained with Feulgen. Each slide was examined independently by two observers (M. F. F. and M. N. R.). Various tissues were studied in each specimen but squamous epithelial cells were selected for routine counting, 100 suitable nuclei being studied in each case.

Six fresh benign cystic teratomas were subjected to chromosome analysis using Harden's method.²⁶ From each specimen, several representative pieces of tissue were taken. Each was divided into two parts, one for histologic study and the other for tissue culture, in order to allow accurate correlation between the nature of the tissue and its chromosome constitution. Small fragments of tissue were grown as explants in plasma clots and chromosomes were prepared from subcultures.

Results

Sex chromatin. In the 187 specimens we studied, the percentage of cells with demonstrable sex chromatin varied between 23 and 87 per cent with a mean of 56 per cent. In several tumors, an occasional cell appeared to show double sex chromatin, but in no case was this the predominant cell type. These findings indicate that our specimens were chromatin positive as in normal female tissues.

Chromosome analysis. The distribution of chromosome counts is shown in Table I. A total of 155 cells were counted, of which 123 had 46 chromosomes, 23 had counts in the hypoploid range and 8 cells were hyperploid. Endoreduplication was noticed in one cell from Case No. 259. Visual analysis revealed a normal karyotype in all 6 cases (Fig. 1). Case No. 280 is seen to have a minor structural variation, two members of the D group having marked enlargement of the short arms. This is unlikely to be of significance and is regarded as a normal variant.²⁷ All of these 6 cases show a normal female, XX, sex chromosome complement.

Table I. Distribution of chromosome counts in female benign cystic teratomas

Case No.	Total	44	44	45	46	47	48	48
194	30	—	3	1	26	—	—	—
195	37	—	—	—	34	1	2	—
246	24	3	1	1	18	—	—	1
251	4	—	—	—	4	—	—	—
259	17	1	1	1	11	1	1	1
280	42	5	2	4	30	—	—	1
Total	154	9	7	7	123	2	3	3

Table II. Sex chromatin studies in female gonadal teratomas

Author	No. of cases	Chromatin positive	Double sex chromatin	Chromatin negative	Doubtful
Hunter and Lennox ⁵	10	10			
Tavares ^{6, 7}	32	32			
Moore and Barr ⁸	4	4			
Levij ⁹	20	20			
Sohval and Gaines ¹⁰	10	5		5	
Mancini ¹¹	12	12			
Brüning ¹²	50	21		9	20
Riviere ¹³	64	64			
Maltzeff and Sacco ¹⁴	4	4			
Myers ¹⁵	47	47			
Hienz ¹⁶	24	17		4	3
Theiss, Ashley, and Mostofi ¹⁷	20	20			
Taylor ¹⁸	12	10	1	1	
Girelli and Rigoli ¹⁹	12	10		2	
Talvalkar ²⁰	12	12			
Present investigation	187	187			
Total	520	475	1	21	23

Comment

The results of previous studies of sex chromatin in ovarian teratomas, together with our own data, are summarized in Table II. The vast majority are chromatin positive,

but a few have been reported as chromatin negative. Undoubtedly, some of these anomalous results were derived from material which was unsuitable for the demonstration of sex chromatin.¹⁰ Furthermore, in any highly

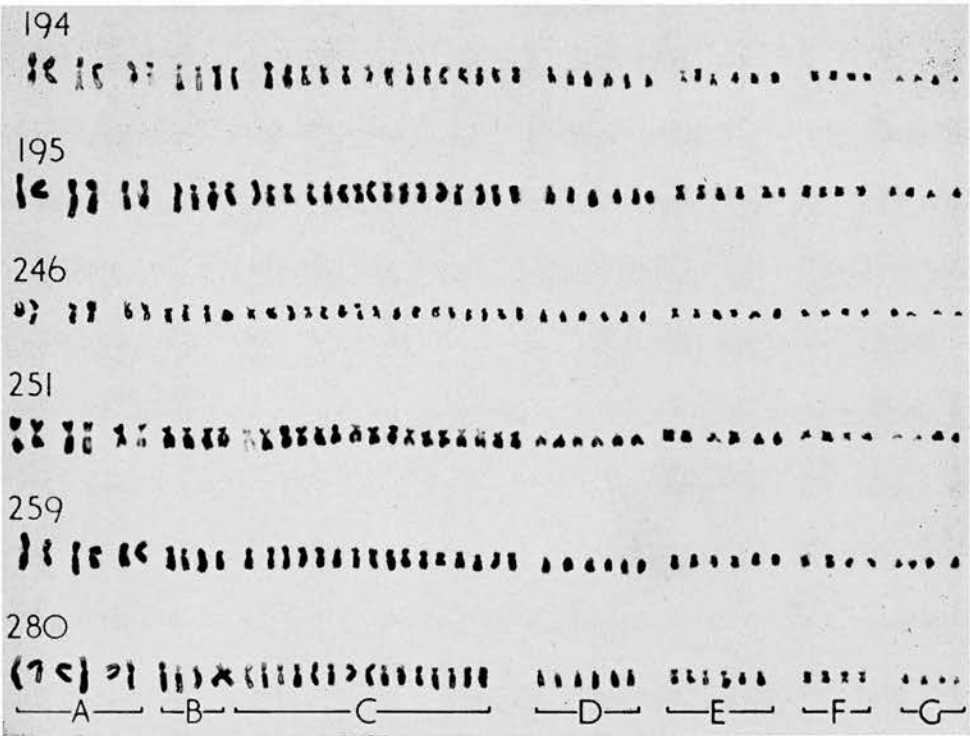


Fig. 1. Karyotypes in 6 benign cystic ovarian teratomas.

malignant tissue there may be anomalous sex chromatin and this may explain some of the chromatin-negative malignant ovarian teratomas.²² There is also one well-documented benign ovarian teratoma which contained double sex chromatin.¹⁸ Similar results from studies on extragonadal teratomas in females have recently been reported. It seems likely that whereas the majority of female teratomas have a "female" sex chromatin pattern, a few may show anomalous nuclear sex.

The results of sex chromatin studies in male teratomas, both testicular and extragonadal, have also been reviewed.^{21, 28} Here, the findings are even more striking insofar as 40 per cent are chromatin positive, while a few cases of sex chromatin mosaicism and double sex chromatin have also been recorded. Many testicular teratomas are highly malignant and this may invalidate the interpretation of some of these results. Nevertheless, anomalous sex chromatin has been clearly demonstrated in benign male teratomas and it is impossible to dismiss all of these results simply as artifacts.

These findings suggest that there may be some discrepancy between the nuclear sex of the host and that of the teratoma, and so cast doubt on the concept of the simple neoplastic origin of these tumors and raise again the possibility of their origin from germ cells. One simple explanation would be that these anomalous tumors may arise in hosts with similarly abnormal sex chromatin. Teratomas have been described in males with Klinefelter's syndrome,²¹ but it has been firmly established that the majority of teratomas occur in hosts with normal sex chromatin.^{15, 29-31}

The most plausible theories of teratogenesis from germ cells are based on hypothetical mechanisms of parthenogenesis.

1. Normal haploid gametes may fuse to form diploid cells.⁵ In the female, the fusion of haploid, 23X, cells would invariably produce chromatin-positive diploid cells, 46XX. In the male, the presence of both X-bearing, 23X, and Y-bearing, 23Y, cells could combine to form diploid cells with XX, XY, or

YY sex chromosome complement in the ratio of 1:2:1. If YY cells are nonviable, this ratio becomes XX:XY = 1:2.

2. Reduplication of haploid gametes could also produce diploid cells.⁶ In the female this would invariably produce chromatin-positive cells, 46XX. In the male, it would produce equal numbers of chromatin-positive cells, 46XX, and chromatin-negative cells, 46YY. This theory implies that cells with a YY sex chromosome complement are viable. Data derived from the ratio of chromatin-positive to chromatin-negative male teratomas are still inconclusive in favoring either of these hypotheses.²¹

3. Meiotic nondisjunction may be followed by the fusion of aneuploid gametes.³² By such a mechanism, chromatin-negative teratomas may have a 45XO chromosome constitution, chromatin-positive male teratomas have 47XXY, and doubly chromatin-positive teratomas have 47XXX.

Other mechanisms such as failure of reduction division or polyploidy are also possibilities. There are undoubted difficulties to all of these parthenogenetic theories. The most serious problem is that meiosis is thought to occur in males only after puberty,³³ while the first meiotic division in oogenesis occurs during intrauterine development but is arrested at the diplotene stage.³⁴

It is clear that these hypothetical suggestions can be elucidated only by full chromosome analysis. So far, Galton and Benirschke²³ have studied one malignant ovarian teratoma, Harnden²⁵ has analyzed one benign solid ovarian teratoma, and Corfman and Richart²⁴ have studied six benign ovarian cystic teratomas. Our own report adds a further 6 benign tumors. All of these have proved to have a normal diploid female karyotype, 46XX.

This evidence excludes the possibility that teratomas may themselves be haploid. Furthermore, they have a normal karyotype and do not have any autosomal anomaly. This latter possibility was raised by the finding of an extra autosome in an amorphous acardiac fetus.³⁵ The features of disordered organo-

genesis in this monozygotic twin were very similar to those of some teratomas, and it seemed possible that a similar anomaly may be partly responsible for the chaotic disorganization of teratomas.

These studies do not provide an answer to the origin of anomalous sex chromatin in some teratomas since all our specimens were normally chromatin positive. Further information is more likely to come from the

chromosome analysis of testicular teratomas, and the study of chromatin-positive male tumors should give definite evidence as to the histogenesis of teratomas.

We wish to acknowledge the skilled assistance of Mrs. Sheila Christie and Miss Aileen Ross in the cytogenetic studies, and Professor R. J. Kellar for access to the collection of ovarian tumors in the Department of Obstetrics and Gynaecology.

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PRIMARY MESODERMAL MIXED TUMOURS IN THE OVARY

A Report of Two Cases

BY

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PRIMARY mesodermal mixed tumours of the ovary are exceedingly rare, but they are of interest because of their possible origin in foci of endometriosis (Willis, 1960). Two cases were encountered in a series of 418 primary malignant ovarian tumours.

Case 1. This patient, a nullipara, aged 59 years, presented with ascites and a twisted tumour of the left ovary, more than 20 cm. in diameter. A total hysterectomy with bilateral salpingo-oophorectomy was carried out. An extensive right pleural effusion, present before the operation, cleared gradually after operation. On pathological examination, the uterus was found to be normal and the endometrium showed a mild degree of proliferative activity. The right ovary and the tubes showed no remarkable features. The sections available from the tumour showed in some the appearance of an old adenomyoma (Fig. 1A) and in the others a picture of carcino-sarcoma with islands of degenerate chondrous tissue (Fig. 1B). Haemorrhage and necrosis were marked in some parts, possibly partly due to the torsion of the tumour.

In this case, the origin of the primary mesodermal mixed tumour from the associated endometriosis was probable, but in the absence of the original specimen it could not demonstrate a definite transitional zone between the two lesions.

Case 2. This patient, a nullipara, aged 64 years, had a mobile solid ovarian tumour. Total hysterectomy with bilateral salpingo-oophorectomy was performed. The tumour was apparently confined to the ovary, but the patient died four months later with

ascites and liver enlargement. No autopsy was performed. Pathological examination of the operative specimen showed a normal uterus with an atrophic endometrium. The cervix was the seat of superficial inflammation. Both tubes and the right ovary showed no abnormal features. The left ovarian tumour showed several cystic areas containing blood stained fluid but was largely solid. Microscopical examination showed the presence of malignant epithelial elements as well as a richly cellular stroma. The epithelial elements showed mostly a well differentiated glandular pattern (Fig. 2A). In the stroma, broad irregular bands of fibres were interlacing in various directions. In the interstices, collections of large cells, rounded, oval or tadpole-like, and with abundant strongly eosinophilic cytoplasm could be seen which were interpreted as rhabdomyoblasts (Fig. 2B). Further sections stained with Heidenhain's iron haematoxylin demonstrated cross striated muscle fibres in several areas (Fig. 2C).

COMMENT

Ovarian tumours resembling the mesodermal mixed tumours of the uterus are very rare. A search of the literature is hampered by the multitude of names under which such tumours may have been reported. The case of Katsunuma *et al.* (1959) was stated to be the first case reported under the title of mixed mesodermal tumour of the ovary. Another case was reported by Edghill *et al.* (1967). Other authors have mentioned similar cases but without giving full details (Willis, 1960; Sternberg, 1963). Cases of ovarian carcino-sarcoma, probably variants of this group, have also been reported (Tuthill, 1938; MacFarlane and Pritchard, 1954; Marcella and Cromer, 1959). Other cases have been reported under the title of rhabdomyosarcoma (Sandison, 1955; Payan, 1965).

In the diagnosis of a mesodermal mixed tumour of the ovary, care should be taken to exclude the possibility of a uterine primary growth. Taylor (1958) commented on three instances of reputed tumours having the histological features of mesodermal mixed tumour, in none of which the uterus was removed so that the possibility of ovarian metastatic growth could not be excluded. Care should also be taken not to mistake cases of teratoma for these tumours. Mesodermal mixed tumours are monodermal while teratomas are tridermal. The presence of ectodermal and endodermal teratomatous elements should be excluded.

Willis (1960) stated that, in the ovary, these tumours arise from foci of endometriosis. The three cases of ovarian carcino-sarcoma, already referred to, were all described in relation to endometriosis, and this was possible in the first of the present cases.

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RUPTURE OF LOWER UTERINE SEGMENT SCAR DURING ABORTION AT TWENTY-TWO WEEKS

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RUPTURE of a lower segment Caesarean scar is uncommon during pregnancy and very rare during abortion at the 22nd week.

The patient was 23 years old. Her first three pregnancies ended with spontaneous vaginal deliveries in 1960, 1961, and 1962, the birthweights being between 4 pounds 8 ounces (2,025 g.) and 5 pounds 0 ounces (2,530 g.). In 1964 labour began at term in her fourth pregnancy and because of an uncorrectable transverse lie, lower segment Caesarean section was carried out. The operation was described as routine, the lower uterine segment being closed in two layers with continuous No. 1 chromic catgut.

The puerperium was uneventful, and the only rise of temperature afterwards was to 99° F. on one occasion on the third postoperative day. She failed to attend the postnatal clinic. Her fifth pregnancy in 1965 was normal and ended with a spontaneous vertex delivery of an infant weighing 5 pounds 5 ounces (2,390 g.) after eleven hours labour. Again the puerperium was normal.

On the 11th September, 1966, the patient was admitted as an emergency case. She gave a history of 22 weeks amenorrhoea, vaginal bleeding for 12 weeks, heavier for one week, and abdominal pains for six hours. During the previous four weeks the patient had had some abdominal discomfort. She denied interference but had not sought medical advice, hoping that she would abort.

Her mucous membranes were pale, the blood pressure was 130/80 mm. Hg, pulse rate 100 per minute and the temperature normal. The uterine fundus was palpable just below the umbilicus; it was tender and contracting. Vaginal examination showed the cervix to be dilating and foetal parts could be felt in the cervical canal. The haemoglobin concentration was 8.4 g. per 100 ml. The patient was given pethidine and appeared to go out of labour. Over the next 12 hours the pulse rate rose to 130, the uterus became increasingly tender and tenderness developed over the lower abdomen. The serum electrolytes were normal and blood cultures were taken. The first of four bottles of blood was given and tetracycline was added to the drip. However, the patient's condition deteriorated further, the pulse rate rose to 140, the respiratory rate to 50, the abdominal tenderness became widespread with marked rebound tenderness and the patient complained of shoulder pain. It was now obvious that there was intraperitoneal haemorrhage. Laparotomy showed that there were approximately 1,500 ml. of blood in the peritoneal cavity. The lower segment incision had ruptured and the placenta and the upper half of the foetus projected into the peritoneal cavity. The foetus and placenta were removed. The bladder was very adherent and was opened during dissection. This incision was closed in layers and as the bladder could not be freed readily, subtotal hysterectomy was performed. This was done after both internal iliac arteries had been ligated in continuity. A tube drain was passed into the vagina through the cervix before the abdomen was closed. The bladder was drained continuously for seven days. Recovery was uneventful.

Histological examination showed no significant abnormality of the myometrium.

The correct diagnosis might have been made earlier in this case had it not seemed so unlikely with regard to the period of gestation.

MALIGNANT TRANSFORMATION IN OVARIAN ENDOMETRIOSIS

BY
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MALIGNANT TRANSFORMATION IN OVARIAN ENDOMETRIOSIS

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It is now forty years since Sampson (1924, 1925) first pointed out the possibility of malignant transformation in ovarian endometriosis. However, its frequency and clinical and pathological characteristics remain widely unrecognized. Few studies have been made and most of the literature on the subject has been limited to the reporting of isolated cases.

Recent interest in this problem has been aroused by the more general realization among pathologists of the frequency with which endometrial-like adenocarcinoma is encountered among cases of primary ovarian carcinoma (Long and Taylor, 1964). The General Assembly of the International Federation of Gynaecology and Obstetrics at Mar del Plata in September 1964 adopted a recommendation to introduce a new group, termed "endometroid", into the histological classification of the common primary epithelial tumours of the ovary, because the frequency of occurrence of tumours so designated was considered to warrant the status of a separate group.

MATERIAL

For this study we reviewed the histological material in the Department of Obstetrics and Gynaecology of Edinburgh University, for a period of ten years, 1954-1963. Among the total of 62,938 cases reported on during this period there were 637 cases of ovarian endometriosis and 1,734 ovarian tumours. In 45 of the cases of endometriosis, the diagnosis was only presumptive, due to the absence of well-defined endometrial elements. The slides of the remaining 592 cases were reviewed for evidence of pre-malignant or early malignant changes. Of the 1,734 ovarian tumours 418 were primarily malignant tumours, and these were critically examined for evidence of a possible or definite origin in endometriosis.

RESULTS

Ovarian Endometriosis

Atypical epithelial activity was encountered in four endometrial cysts, without any evidence of stromal invasion. Heaping up in layers, cellular pleomorphism, variations in the size of the nuclei, nuclear hyperchromatism and mitotic figures were noted to variable degree in these cases. The ages of the four patients were 45, 48, 55 and 60 years.

Post-menopausal benign hyperplasia was also observed in two cases of ovarian endometriosis associated with a granulosa-theca cell tumour in the opposite ovary.

The association of endometriosis with an ovarian tumour in the same or the opposite ovary was observed in 52 cases. In 21 of these the tumour was malignant. In seven cases the endometriosis and the malignant tumour were encountered in the same ovary. The malignant tumours in these seven cases were two adenoacanthomas, four endometroid adenocarcinomas and one mesodermal mixed tumour. Definite evidence for an origin in endometriosis could be established for the two adenoacanthomas and for two endometroid carcinomas.

Primary Malignant Ovarian Tumours

In the survey of our 418 primary malignant ovarian tumours, we classified 52 cases as having a histological appearance similar to that of malignant tumours of the uterine endometrium. Of these 12 were adenoacanthomas, 38 were adenocarcinomas and two were mesodermal mixed tumours. These malignant "endometroid" tumours thus accounted for about 12 per cent of all primary malignant tumours of the ovary in our series. Unfortunately the uterus was not available for examination in 17 of these cases—three adenoacanthomas and 14 adenocarcinomas.

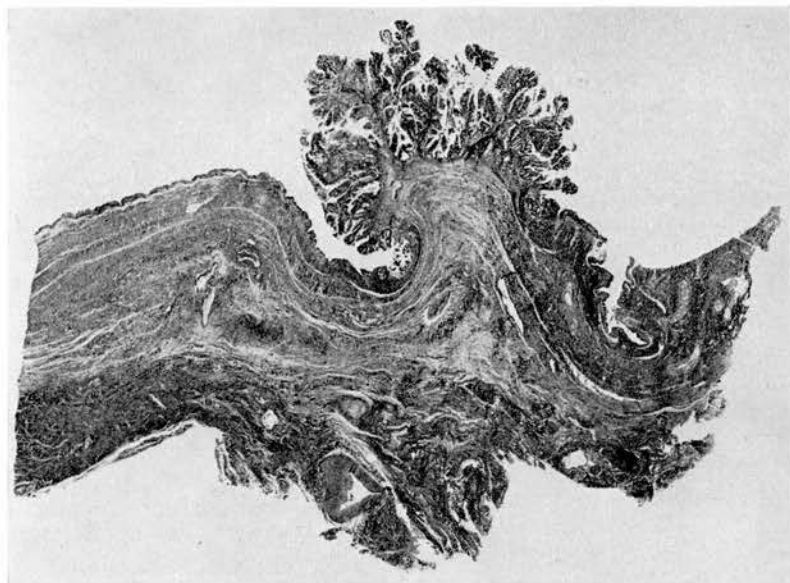


FIG. 1

Case 1. Papillary malignant growth in an ovarian endometrial cyst ($\times 5$).

The possibility of a metastatic spread from a primary uterine tumour cannot be excluded on histological review in these cases.

The association of benign endometriosis with these malignant endometroid tumours was as follows. Two cases of adenoacanthoma and two cases of endometroid carcinoma had their origin in benign endometrial cysts. In two cases of endometroid carcinoma and in one case of mesodermal mixed tumour endometriosis was encountered in the same ovary as the tumour but no transitional areas could be satisfactorily demonstrated. The opposite ovary was available for examination in 22 of the other cases. In six of these endometriosis was encountered and in one case it showed pronounced cystic hyperplasia.

CASE REPORTS

A brief report of the four cases of malignant transformation in ovarian endometrial cysts will now be given.

Case 1: The patient was 51 years old, nulliparous and two years postmenopausal. A diagnosis of uterine fibromyomata and ovarian

endometriosis was made. A total hysterectomy with bilateral salpingo-oophorectomy was performed and a localized papillary adenocarcinoma in an endometrial cyst was found (Fig. 1). Eighteen months later the patient returned with abdominal pain, a lump and urinary frequency. Laparotomy was carried out and a bilocular completely encapsulated cyst about 10 cm. in diameter was removed from the site of the right broad ligament. During removal one loculus ruptured with escape of chocolate brown fluid. Gross pathological examination showed intracystic papillae and microscopical examination showed adenoacanthoma. An interesting feature was the apparent malignant nature of both the glandular and squamous elements in this tumour (Fig. 2). Although no definite benign endometrial tissue was encountered in the sections available from this second tumour, the cyst was partly lined by a broad zone of pseudo-xanthoma cells (Fig. 3) and old haemorrhages were abundant in the stroma. We interpret the second tumour in this case as representing malignant transformation in an endometrial lesion left from the previous operation, rather than a recurrence of the first localized growth.

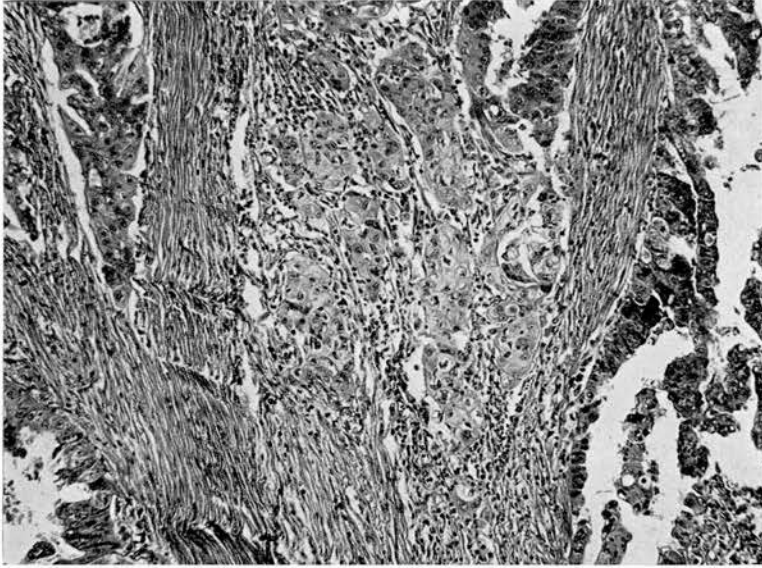


FIG. 2

Case 1. Malignant squamous component in the adenoacanthoma ($\times 110$).

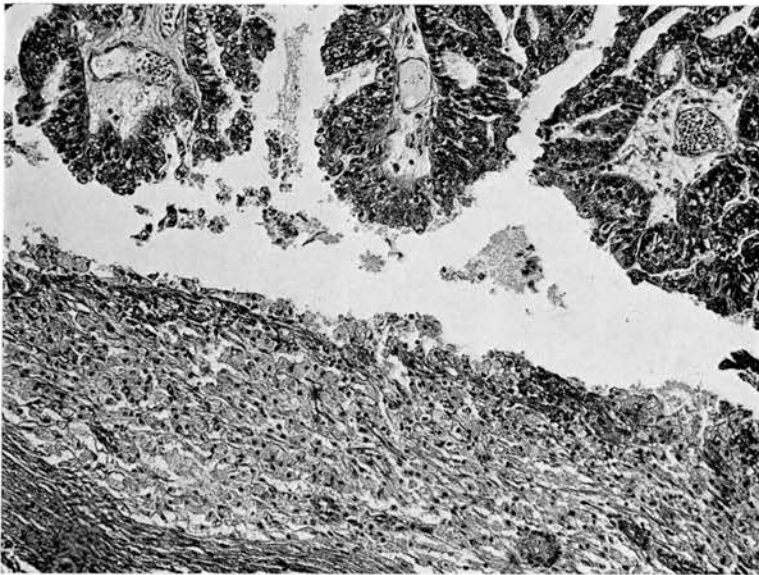


FIG. 3

Case 1. A layer of pseudoxanthoma cells forming part of the lining of the malignant endometrial cyst ($\times 110$).



FIG. 4

Case 2, Papillary adenoacanthoma in an endometrial cyst ($\times 95$).

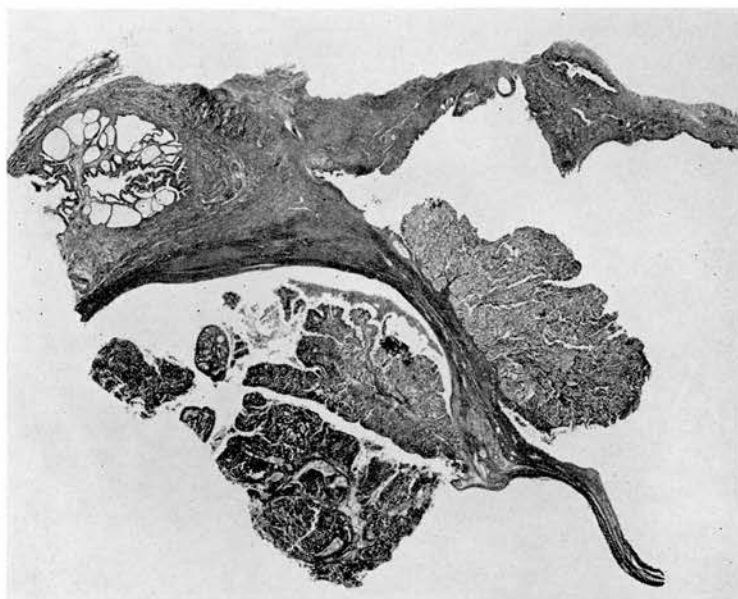


FIG. 5

Case 3. Papillary malignant areas in an endometrial cyst. An area of cystic hyperplasia is shown in the upper left corner ($\times 4$).

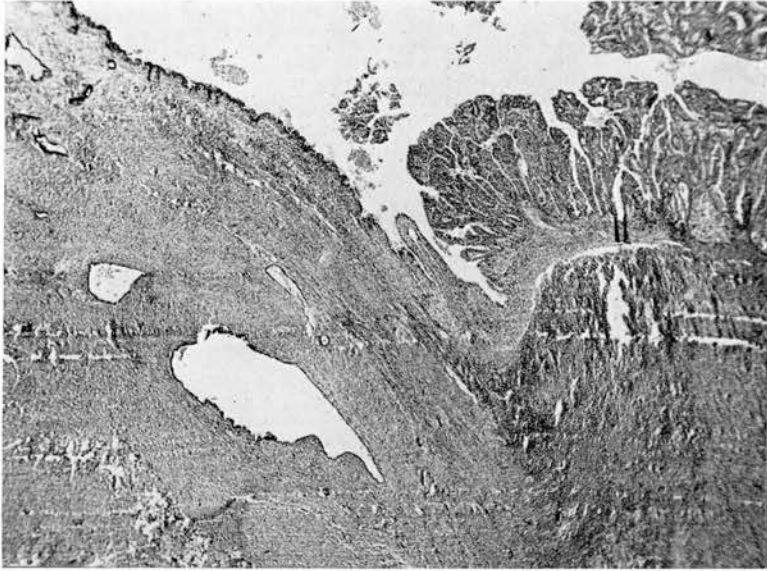


FIG. 6

Case 4. Benign endometriosus running into and continuous with the malignant epithelium ($\times 25$).

Case 2: The patient was 47 years old and was operated upon for an asymptomatic abdominal mass. An adherent right ovarian cyst was found as well as an anterior wall fibroid. The cyst ruptured during removal with the escape of brownish stained fluid and pieces of friable tissue. Subtotal hysterectomy with bilateral salpingo-oophorectomy was carried out and the patient received a post-operative course of deep X-ray therapy. Three years later, when the patient was last seen, there was no evidence of any recurrence. Pathological examination of the cyst showed a localized papillary adenoacanthoma (Fig. 4). The rest of the cyst wall showed some atrophic endometrial glands but the lining epithelium was largely replaced by a broad zone of pseudoxanthoma cells. The other ovary contained a small blood cyst.

Case 3: An unmarried woman, 46 years old, presented with acute abdominal pain and a tender pelvi-abdominal mass. Laparotomy showed that the peritoneal cavity was filled with a light brown opaque fluid, which had come from a ruptured left ovarian cyst. The cyst was thick walled, adherent and still distended to about the size of a large grapefruit by some brown

thick fluid. Pathological examination of the removed cyst showed that it was an endometrial cyst with areas of endometrial cystic hyperplasia and areas of papillary adenocarcinoma (Fig. 5).

Case 4: An unmarried woman, 27 years old, presented with recurrent attacks of hypogastric pain and an enlarging abdominal lump. A left ovarian cyst, together with the uterus and the right ovary were removed. The cyst was about 15 cm. in diameter and contained copious amounts of dark fluid. Multiple small papillomata were seen to fill almost half the cyst cavity. Microscopical examination showed a papillary endometroid carcinoma arising from and continuous with the benign epithelial lining of an endometrial cyst (Fig. 6). The opposite ovary was the seat of endometriosis.

DISCUSSION

As Sampson (1925) pointed out, two methods of studying malignant transformation in ovarian endometriosis are possible—either to study cases of benign endometriosis in an endeavour to find early malignant changes in them or to study cases of ovarian carcinoma to ascertain whether the carcinoma could have arisen in misplaced endo-

metrial tissue. We have used both methods. To prove beyond doubt that an ovarian carcinoma arises from pre-existent endometriosis the criteria as suggested by Sampson (1925) and Scott (1953) must be satisfied. Such direct evidence may only be available in early cases; cases detected later may have all traces of their origin obliterated by spread of the malignant growth. Indirect evidence for an origin of a malignant tumour in endometriosis may be its histological similarity to endometrial growths in the uterus. Malignant ovarian tumours of endometroid type are not infrequent. In our material they accounted for about 12 per cent of all primary malignant tumours of the ovary. We included in this group cases of adenoacanthoma, endometroid carcinoma and the rare cases of primary mesodermal mixed tumours. However, it is also possible that these tumours can arise directly from the ovarian serosa, which may retain the embryologic potential of the coelomic epithelium to differentiate into various tissues, including uterine epithelium (Long and Taylor, 1964). In the present state of our knowledge the incidence of malignant transformation in ovarian endometriosis can therefore only be given as lying somewhere between a minimum which only includes the definite cases and a maximum which includes all the cases showing a histological pattern compatible with an endometrial origin.

REVIEW OF REPORTED CASES

We have analyzed the cases of definite malignant transformation in ovarian endometriosis which have been reported in an attempt to define the clinical and pathological features which were possibly responsible for their early detection, when their relation to benign endometriosis was still intact. For this purpose, we added 48 other cases from the literature to our own 4 cases. (Sampson, 1925, case 5; Tuthill, 1938; McCullough *et al.*, 1946; Teilum, 1946; Novak, 1947; Kuzma, 1947, case 1; Miller *et al.*, 1947; Rauramo, 1947; Moss and Runals, 1948; Corner *et al.*, 1950, cases 4 and 5; Bacher and Hertzog, 1951; Scott, 1953, case 3; Fredrikson, 1953, case 1; Hunter and Klein, 1954, cases 1 and 2; Dockerty, 1954, 2 cases; Postoloff and Rodenberg, 1955; Kumar *et al.*, 1955; Weinrod *et al.*, 1956; Thompson, 1957, cases 3, 5, 7, 10, 14

and 17; Greene and Enterline, 1957, cases 1 and 2; Ferreira and Clayton, 1958; Wade, 1960; Burt and Emson, 1961, 3 cases; Concannon *et al.*, 1962; Dockerty, 1962, cases 5, 6, 7, 8 and 9; Freedman *et al.*, 1964; Gray and Barnes, 1965, cases 1, 2, 4 and 5; Ridley, 1966; Plate 1966; Eastman *et al.*, 1966). This is not claimed to be a comprehensive review of all reported cases, though it probably includes all acceptable cases in the English literature.

Clinical features

The frequency of the leading symptoms was as follows:

Pain	23 cases
Lump or abdominal enlargement	19 cases
Uterine bleeding	12 cases
Urinary symptoms	6 cases
Asymptomatic	6 cases
Not stated	2 cases

Pain was the most presenting symptom. Judging from the gross appearance of the endometrial cysts in these cases, in which the malignant growth was quite commonly small, the pain was caused by bleeding from the endometrium inside the cyst. The pain in these cases was frequently more or less continuous and was different from the periodic menstrual-related pain typical of benign endometriosis. When intermittent attacks were experienced they were not usually related to menstruation, except in their early stages. In some cases the pain began after the menopause. These observations suggest that the possibility of malignant transformation should be considered in case of a patient known to have endometriosis if the typical periodic pattern of pain changes, if the pain increases abruptly in severity or if attacks of pain start after the menopause.

In some cases the cyst enlarged to form an abdominal lump or to cause a feeling of abdominal enlargement without causing any marked pain. Commonly, however, the patient complained of both pain and the feeling of a lump. The abdominal lump may be noticed to develop rapidly (Weinrod *et al.*, 1956). The enlargement of the endometrial cyst was not caused by the bulk of the malignant growth which was almost invariably small in these cases but by distension due to bleeding from the malignant endomet-

rium. The detection of an enlarging mass in a patient known to have endometriosis may direct attention to the possibility of malignant transformation (Dockerty, 1962, case 7).

In one of our cases, and in two other cases (Teilmann, 1946, Dockerty, 1962, case 5) the patient presented with acute abdominal pain due to rupture of an endometrial cyst that had undergone malignant transformation. Acute spontaneous rupture of an endometrial cyst is a rare condition (Koskela, 1964). Pregnancy is the only significant factor predisposing to rupture which has been hitherto described (Scott, 1944), but malignant transformation is another possible cause.

The three features of pain, an enlarging lump and spontaneous rupture may all be due to intracystic bleeding from the malignant endometrium, and may sometimes draw attention to these cases at an early stage.

In these cases uterine bleeding often occurred. Out of 30 cases in which the endometrial histology was reported the endometrium was described as hyperplastic in 15 cases. We have also seen recently a case of primary adenoacanthoma of the ovary with the benign proliferative uterine endometrium showing few areas of squamous metaplasia.

Gross Appearances

The malignant endometrial cysts commonly showed one or more of the following gross pathological features:

Papillary areas	22 cases
Solid areas	13 cases
Abnormal large size	11 cases
Spontaneous rupture	3 cases

The presence of any of these features in an endometrial cyst should raise suspicion about malignant transformation. The tendency of the growth to project inside the cyst explains the intracystic bleeding already discussed. A large size is very unusual for benign endometrial cysts; the largest encountered by Sampson was 15 cm. in diameter (Sampson, 1925). Malignant endometrial cysts, on the other hand, may reach very large sizes, apparently due to recurrent bleeding from the endometrium. The cyst reported by McCullough *et al.* (1946) contained about 4,000 ml. of dark brown fluid, and case 14 of Thompson (1957) contained 2,700 ml.

Microscopical Picture

The types of tumour in cases reviewed were as follows:

Adenoacanthoma	25 cases
Adenocarcinoma	25 cases
Squamous cell carcinoma	1 case
Carcinosarcoma	1 case

The frequency of the adenoacanthoma in these cases is probably more apparent than real. The adenoacanthoma is commonly a slowly growing tumour (Thompson, 1957) so that its relation to an endometrioma may remain intact whereas a more rapidly growing adenocarcinoma is liable to overgrow the tissue of its origin so that its relation to endometriosis may be missed. In our cases of primary malignant ovarian tumours, the ratio of cases of adenoacanthoma to endometrioid carcinoma was 1:3; if only the cases which definitely arose in endometriosis are considered, the ratio is 1:1.

SUMMARY

Malignant transformation in ovarian endometriosis has been studied by reviewing 592 cases of ovarian endometriosis and 418 primary malignant ovarian tumours. Anaplastic changes were encountered in four benign endometrial cysts. Malignant tumours histologically similar to endometrial growths were encountered in 52 cases. Of these, only four could be shown to have a definite endometriotic origin. The clinical and pathological characteristics of these four cases and of 48 other cases from the literature have been reviewed.

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THE RELATIONSHIP BETWEEN OVARIAN
TUMOURS AND INTERSEX STATES WITH
SPECIAL REFERENCE TO THE
DISGERMINOMA AND ARRHENOBLASTOMA

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THE RELATIONSHIP BETWEEN OVARIAN TUMOURS AND INTERSEX STATES WITH SPECIAL REFERENCE TO THE DISGERMINOMA AND ARRHENOBLASTOMA

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THE association between intersex states and ovarian tumours, particularly the disgerminoma and arrhenoblastoma, has at least two points of interest. It has immediate relevance to our understanding of the nature and histogenesis of these tumours, and it is of practical importance to the clinician in his management of intersex states.

In the first three decades of this century many clinical and pathological studies of this problem were reported and this epoch may be said to have ended with a more or less general acceptance of Robert Meyer's views. In a series of publications (1931a, 1931b) he drew a sharp distinction between two types of ovarian tumour which are associated with intersex states. In one variety, which he called disgerminoma, he postulated that the intersex state favoured the occurrence of the tumour, but the tumour was in no way responsible for the abnormal sex development. The other type he called arrhenoblastoma and suggested that this tumour was itself directly responsible for virilisation so that the intersex state was reversible when the tumour was removed.

Meyer wrote disgerminoma with the prefix "dis" meaning "both", implying that the germinal cells from which this tumour arises are present in both the testis and ovary. Most later authors have used the spelling with the prefix "dys", implying that the germinal cells from which the tumour arises have undergone an error of development or differentiation. For historical reasons we have preferred to use Meyer's original spelling.

The introduction of methods of analysis of chromosomes and sex chromatin has led to a better understanding of intersex states and this new information has raised several problems which appear to complicate Meyer's concepts. Morris (1953) suggested that some tumours which had been previously reported as arrhenoblastoma may in fact have been testicular tumours occurring in association with the syndrome of testicular feminization in genetic males. Carpentier *et al.* (1956) went further and postulated that some other ovarian tumours, particularly disgerminoma and chorioncarcinoma, may also be derived from testicular tissue. Overzier (1963) considered that an intersex state should be suspected in every patient with a disgerminoma. Scott (1965) has gone so far as to suggest that an intersex state may underlie the development of all disgerminomas in the female, and he advocates a return to the term used by early French pathologists, seminoma ovarii. He implies that the testicular seminoma and the ovarian disgerminoma are identical and postulates that the presence of either of these tumours is dependent on the presence of an XY cell line in the gonad.

It seems timely to attempt a synthesis of both the cytogenetic and pathological evidence on this problem. In this communication we describe the results of a combined cytogenetic and histological study of 5 cases of disgerminoma and 5 cases of arrhenoblastoma, and present a critical analysis of the relevant literature.

MATERIAL AND METHODS

Nine of these cases were obtained as a result of the review of more than 2,000 ovarian tumours collected in the laboratories of this department over the last ten years. Histological sections of one dysgerminoma (case 5) came from the pathology department of the Royal Hospital for Sick Children, Edinburgh. The histopathology of the original slides was re-assessed. Sex chromatin was studied in slides stained both with haematoxylin and eosin and with Feulgen. The clinical records of each patient were reviewed, and whenever possible the patient was recalled for assessment of sex chromatin from a buccal smear, and for chromosome analysis of peripheral blood leucocytes (Moorhead *et al.*, 1960).

Case 1. The patient was 59 years old. She married at the age of 46 and had no children. Her periods were regular until the menopause at 52 years. Seven years later she presented with postmenopausal bleeding. A pelvic tumour was detected, and total hysterectomy and bilateral salpingo-oophorectomy were performed. Fifteen months later she died with a recurrent abdominal mass.

The left ovary was replaced by a dysgerminoma and dermoid cyst, and the uterus contained multiple fibroids. Normal ovarian tissue could not be found in the sections made from the tumour, but the other ovary was normal. The squamous epithelium of the dermoid cyst, the healthy ovary and the uterine fibro-muscular tissue proved to be chromatin positive. Some of the cells of the dysgerminoma appeared to be chromatin positive but in general these nuclei were unsuitable for analysis. However, the fibroblasts of the stroma of the tumour were definitely chromatin positive.

Case 2. The patient was 16 years old and unmarried. Her periods began at the age of 15 and were subsequently irregular. Examination revealed a large pelvic tumour, and the urine showed a high titre of chorionic gonadotrophin. At first she was thought to be pregnant but a laparotomy was subsequently performed. A large right ovarian tumour was found, and a total hysterectomy and bilateral salpingo-oophorectomy performed. The clinical and endocrinological aspects of this unusual case have already been reported (Hobson and Baird, 1966).

The ovarian tumour was a dysgerminoma, and a corpus luteum was found in the right ovary immediately adjacent to the tumour cells. The other ovary was normal and the endometrium was in the secretory phase. The healthy ovary, myometrium and tumour stroma were chromatin positive. The tumour cells were unsuitable for the demonstration of sex chromatin. A buccal smear was chromatin positive, and chromosome analysis of peripheral blood leucocytes revealed a normal female karyotype, 46/XX.

Case 3. The patient, who was 18 years old and single, presented with abdominal distension, hypertension, proteinuria and pretibial oedema. At first she was thought

to be pregnant with pre-eclamptic toxæmia, but pelvic examination revealed a swelling discrete from the uterus. Subsequent laparotomy showed a tumour of the left ovary and left oophorectomy was carried out.

The ovarian tumour was a dysgerminoma. Nuclei of the tubal epithelium and of stromal fibroblasts of the tumour were chromatin positive, but the tumour cells were unsuitable for analysis. A buccal smear was chromatin positive, and chromosome analysis of peripheral blood leucocytes revealed a normal female karyotype, 46/XX.

Case 4. The patient was 19 years old and unmarried. She had a laparotomy at the age of 7 when she presented with an abdominal tumour. The right ovary contained a large tumour, and right oophorectomy was followed by a course of radiotherapy. At the age of 19 years she was treated for an incomplete abortion.

The ovarian tumour was a dysgerminoma. Although the tumour cells were unsuitable for analysis, the stromal elements were chromatin positive. She had a chromatin positive buccal smear, and chromosome analysis showed a normal female karyotype, 46/XX.

Case 5. The patient was 4 years old and presented with a symptomless abdominal swelling. At laparotomy a left ovarian tumour was discovered and the left ovary was removed.

This tumour was a dysgerminoma with a marked lymphoid reaction. The tumour cells were unsuitable for analysis, but stromal elements were clearly chromatin positive. This patient could not be traced for further cytogenetic investigation.

Case 6. The patient was 16 years old and presented with primary amenorrhoea. There was normal breast development, but infantile external genitalia, and neither axillary nor pubic hair. At laparotomy there was no uterus and the gonads were slightly enlarged and lay on the brim of the pelvis. Both gonads were removed.

The gonads were at first reported to contain bilateral well-differentiated arrhenoblastomas. The capsules of the tumours resembled ovarian stroma but contained no follicles. The tubular elements of the tumours were lined with Sertoli cells but there were no spermatogenic elements. There were occasional collections of Leydig cells. The nuclei of the stromal cells, Sertoli cells and Leydig cells were chromatin negative.

Prior to our investigation this patient had already been found to have a chromatin negative buccal smear and a normal male karyotype, 46/XY (MacGregor, 1966). This is an illustration of the association of a testicular tubular adenoma with the clinical syndrome of testicular feminization in a genetic male.

Case 7. The patient was 18 years old and presented with primary amenorrhoea. At subsequent laparotomy, the uterus was absent and gonads were found to be enlarged. Both gonads were removed.

Again, these gonadal tumours were at first thought to be well-differentiated arrhenoblastomas, and the histological picture was identical to that described in Case 6. The nuclei of both the tumour cells and stromal cells were chromatin negative.

Nuclear sexing and chromosome analysis had been performed prior to our investigation, and the patient

proved to be chromatin negative with a normal male karyotype, 46/XY, (MacGregor, 1966). This case represents another example of the association between testicular tubular adenoma and testicular feminization.

Case 8. The patient was 24 years old. She had been married for four years and had one child aged one year. She presented with secondary amenorrhoea and hirsutism following delivery. At laparotomy she was found to have a left ovarian tumour and this was removed. Following this operation her periods returned, the hirsutism disappeared, and she subsequently had two further pregnancies.

This tumour was an arrhenoblastoma of intermediate to sarcomatous type. The Sertoli cells, Leydig cells and stromal elements of the tumour were chromatin positive. The patient could not be traced for further examination.

Case 9. The patient was 14 years old and presented with abdominal pain and swelling. There was no evidence of virilization and she had not reached the menarche. At laparotomy a large cystic left ovarian tumour was removed. She died with a pelvic recurrence 21 months later.

The tumour was an arrhenoblastoma. Nuclei of the stromal elements of the tumour were chromatin positive, but nuclei of the tumour cells were unsuitable for analysis.

Case 10. The patient, who was married and aged 24, presented with abdominal swelling of recent onset and secondary oligomenorrhoea for four years. At laparotomy, a large right ovarian tumour was removed.

The tumour was a well-differentiated arrhenoblastoma. This histological picture showed a predominantly tubular pattern with a few areas similar to granulosa cell growth. Nuclei of both tumour cells and stromal fibroblasts proved to be chromatin positive. This patient could not be traced for further investigation.

In summary, all five patients with a disgerminoma were phenotypically normal and had a normal female sex chromatin pattern, while three of them had a normal female karyotype. Of the five tumours originally diagnosed as arrhenoblastomas, two proved to be testicular tubular adenomas replacing the undescended testes of genetic males with the testicular feminization syndrome.

DISCUSSION

A. Disgerminomas and Intersex States

Disgerminomas are most commonly found in the ovaries of phenotypically normal women. Pregnancies have both preceded and followed the removal of a disgerminoma (Santesson, 1947), and 23 cases of disgerminoma occurring during pregnancy have been reported (Pecce, 1964). In case 2 of this report we describe a corpus luteum immediately adjacent to tumour cells. The stromal cells in our five cases were

consistently chromatin positive. In our cases the tumour cells were unsuitable for demonstrating sex chromatin, but other authors have found a high incidence of sex chromatin in some disgerminomas (Sohval and Gaines, 1955; Theiss *et al.*, 1960; von Heinz, 1961). Finally, we report here the results of chromosome analysis in three patients with a disgerminoma in each of whom there was a normal female karyotype, 46/XX. The evidence appears to be incontrovertible that disgerminomas usually occur in the ovaries of genetic females.

However, it is commonly believed that there is a significant association between ovarian disgerminomas and intersex states and statements to this effect appear in most of the standard textbooks. The series of 27 disgerminomas in intersex cases collected by Meyer (1925, 1931b) forms the basis for this belief. However, it has not been sufficiently recognized that Meyer used the term disgerminoma to refer to tumours of both the testis and ovary and that his series included no definite cases in female pseudohermaphrodites. In fact, his series includes no example where the gonad which was the site of the tumour could be definitely identified as an ovary, and in only one of his cases was the other gonad shown to be an ovary. Furthermore, this latter case which was originally reported by Moots (1921) was subsequently considered to be an arrhenoblastoma rather than a disgerminoma (Javert and Finn, 1951). In another four of his cases Meyer considered that the contralateral gonad was probably an ovary, but this opinion was not based on histological evidence.

This illustrates a peculiar difficulty in analyzing the cases reported in the literature. The nature of the gonad bearing the tumour is often obscured by the tumour, and the structure of the contralateral gonad is rarely described. For example, Meyer could identify gonadal sex in only 7 out of his 27 cases.

The application of nuclear sexing and chromosome analysis to this problem might be expected to supplement gonadal sexing based on histological evidence. We have, therefore, collected from the literature 36 cases of tumours of the seminoma-disgerminoma type in intersex patients in which one or more of the following pieces of information was available—a histological report on one or both gonads, sex chro-

TABLE I
Tumours of the Disgerminoma-Seminoma Type in Intersexes

Author		Tumour nomenclature	Gonadal sex		Genetic sex	
			Tumour side	Contralateral	Sex chromatin	Chromosome analysis
Unger	(1905)	Disgerminoma	Testis	Testis		
Marion	(1905)	Disgerminoma		Testis		
Zacharias	(1909)	Disgerminoma		Testis		
Skajaa	(1919)	Disgerminoma		Testis		
Polano	(1921)	Disgerminoma	Ovotestis	Ovotestis		
Menetrier <i>et al.</i>	(1922)					
	Case 1	Seminoma	Testis	Testis		
Reverdin	(1923)	Disgerminoma		Ovotestis		
Weyeneth	(1936)	Disgerminoma		Ovotestis		
Essenberg and Feinberg	(1937)	Seminoma	Ovotestis	Ovary		
		(Carcinoma)				
Seegar	(1938)	Disgerminoma	Testis	Testis		
Long <i>et al.</i>	(1941)	Disgerminoma	? Ovary			
Moehlig	(1942)	Seminoma	Testis	Testis		
Corriden	(1949)	Disgerminoma				
Lafferty and Pendergrass	(1950)	Seminoma	Testis	Testis		
Huddleston-Slater	(1953)	Disgerminoma			Negative	
Morris	(1953)	Seminoma	Testis			
		(Disgerminoma)				
Plate	(1953)	Disgerminoma			Negative ¹	46/XY ²
Spielman and Motyloff	(1955)	Disgerminoma		Testis		
Melicow and Uson	(1959)					
	Case 3	Seminoma	Testis	Testis	Negative	
	Case 4	Disgerminoma			Negative	
Stirling	(1959)	Seminoma	Testis	Ovary	Negative	
Teter and Tarlowski	(1960)	Disgerminoma			Negative	
	Case 1	(Seminoma)				
	Case 2	Disgerminoma			Positive	
Botella-Llusia	(1960)	Disgerminoma ³	Ovotestis	Ovotestis	Positive	46/XX
Miller <i>et al.</i>	(1960)	Seminoma	Testis		Negative	XO/XY
		Seminoma	Testis		Negative	XO/XY
Philipp and Stange	(1960)	Disgerminoma			Positive	
Dominguez and Greenblatt	(1962)	Disgerminoma			Negative	45/XO
Goodlin	(1962)	Seminoma	Testis		Negative	XO/XY or (XO/XY/XX)
Reddy and Vittal	(1963)					
	Case 3	Seminoma	Testis	Testis	Negative	
Hauser	(1963)	Seminoma	Testis	Testis		
Halley	(1963)	Seminoma	Testis		Negative	
Lewis <i>et al.</i>	(1963)	Seminoma	Testis		Negative	XO/XY
Teter <i>et al.</i>	(1964)	Disgerminoma			Negative	46/XY
Sohval	(1964)	Disgerminoma			Negative	
Bain and Scott	(1965)	Disgerminoma			Negative	XX/XY

1. Sex chromatin after Carpentier (1956).

2. Chromosome analysis after Scott (1965).

3. Tumour nomenclature after Overzier (1963).

matin analysis, or chromosome analysis of the patient. Fourteen of these tumours were described as seminomas and the other 22 as disgerminomas. Cases reported as embryonal carcinoma, teratocarcinoma and gonadoblastoma have been excluded. A summary of these cases is presented in Table I. This provides information on four specific points.

1. *Nature of the gonad which contained the tumour*

This was reported as follows:

Testis	15 cases
Ovotestis	3 cases
? Ovary	1 case
Not identified	17 cases

The one case where the site of the tumour was identified as an ovary was described by Long *et al.* (1941). This diagnosis was apparently based on the finding of ovarian stroma. However, this cortical type of tissue is not specific to the ovary as it can also be found in ectopic testes (Morris and Mahesh, 1963). Two other features of this case suggest a testicular origin for this tumour. The findings of a deep voice, hirsutism and penile erections suggest that the patient was a male, unless it is assumed that the tumour was actively androgenic. Secondly, calcified concretions were present in the tumour. These are practically unknown in ovarian disgerminomas but are common in the tumours of dysgenetic testes (Teter *et al.*, 1964).

The three cases in which the tumour was found in an ovotestis need further comment. In the case described by Essenberg and Feinberg (1937), the tumour was situated in the testicular part of an ovotestis. In the case originally described by Polano (1921) and reviewed by Meyer (1925 and 1931a), one gonad was replaced by the tumour, while in the contralateral gonad, an ovotestis, Meyer found a focus of disgerminoma cells in the ovarian component. Meyer considered that this was a separate primary disgerminoma arising in ovarian tissue, but it is difficult to exclude the possibility that it may have been a metastatic deposit from the other gonad. In the case of Botella-Llusia (1960) an early focus of what were apparently disgerminoma cells was found between the testicular and ovarian components of one ovotestis. In this case, the origin of the tumour cannot be definitely ascribed to either testis or ovary. One can con-

clude from this data that no case of intersex with a disgerminoma has been reported where the tumour has been proven to have arisen in ovarian tissue. Undoubtedly, the vast majority have arisen in testicular tissue.

2. *Nature of the other gonad.*

The nature of the gonad on the side opposite to the tumour was reported as follows:

Testis	12 cases
Ovotestis	4 cases
Ovary	2 cases
Not identified	18 cases

The two cases in which the other gonad was described as an ovary were those of Essenberg and Feinberg (1937) and Stirling (1959). In both cases the patient was a true hermaphrodite and the tumour developed in a testis.

Two of the cases in which the nature of the gonad was not identified deserve comment. In the case of Corriden (1949) both gonads were replaced by tumour tissue. The gonad contained a disgerminoma, and the author describes an epididymis attached to this tumour. The other gonad was replaced by a typically ovarian type of tumour, a mucinous cystadenoma, and this suggests that this gonad was an ovary. This is most probably an example of true hermaphroditism (Melicow and Uson, 1959). Moehlig's case (1942) was unusual. One gonad, a testis, contained a disgerminoma, and there were two other separate gonads in the pelvis. One of these was removed and found to be a hypoplastic testis. The other gonad was thought to be an ovary, but it was neither removed nor was a biopsy taken.

One can conclude that in many intersex cases with a disgerminoma, the contralateral gonad is a testis. In cases in which the contralateral gonad proved to be either a normal ovary or an ovotestis it is likely that the gonad which was the site of the tumour was either wholly or partly testicular—i.e. all of these patients were probably true hermaphrodites.

3. *Sex chromatin studies.*

There is information about sex chromatin in 19 patients of whom 16 were chromatin negative and 3 were chromatin positive.

The three chromatin positive cases require further comment. In one case (Philipp and Stange, 1960) only 23 per cent of nuclei from a buccal

smear were chromatin positive. This low count is suggestive of some form of chromosomal mosaicism rather than a simple XX sex chromosome complement. Furthermore, the contralateral gonad in this case was rudimentary. In the second case (Teter and Tarlowski, 1960) the tumour replaced one gonad while the contralateral gonad was again rudimentary. The presence of calcification in this tumour suggests that it was testicular in origin. In neither of these two cases was chromosome analysis performed. The third case (Botella-Llusia, 1960) was a true hermaphrodite with bilateral ovotestis. This patient was found to have a normal female karyotype, 46/XX.

One can conclude that the majority of intersex patients with a disgerminoma have been chromatin negative. The three chromatin positive patients which have been reported were probably either true hermaphrodites with testicular tissue or chromosome mosaics in association with dysgenetic gonads. One feature common to all of these patients is likely to have been the presence of testicular tissue.

4. Chromosome analysis.

Chromosome analysis was carried out in 9 cases and the following sex chromosome complements were found:

XY	2 cases
XO/XY	4 cases
XO	1 case
XX/XY	1 case
XX	1 case

The one case with an apparently normal female karyotype has already been discussed (Botella-Llusia, 1960) and shown to be an example of a true hermaphrodite with a 46/XX karyotype. The presence of testicular tissue despite the apparent absence of a Y chromosome has already been well documented (Harnden and Armstrong, 1959). With this exception, no intersex case with a disgerminoma and a normal female karyotype has yet been described. Furthermore, every case has had an XY cell line, with the further exception of one patient with a 45/XO karyotype.

These four observations lead to the conclusion that in patients with an intersex state there has been no clearly documented example in which a disgerminoma has originated in ovarian tissue. Undoubtedly, in the majority of cases it

was shown to derive from testicular tissue. As a corollary to this statement, there is no well-established case of a female pseudohermaphrodite with a disgerminoma, the patients reported being either male pseudohermaphrodites or true hermaphrodites. The liability of a female pseudohermaphrodite or a true hermaphrodite to develop an ovarian disgerminoma is probably the same as in women with normal sex development.

However, tumours other than disgerminoma have occasionally been found in the ovaries of intersex patients. Examples of mucinous cystadenomas (Corriden, 1949; Bani, 1956; Grecham and Fairgrieve, 1960), Brenner tumour (Weed *et al.*, 1947; Stange, 1957), thecoma (Vaughn and Gonzales-Angulo, 1961), granulosa-theca cell tumour (Hauser *et al.*, 1960), and dermoid cyst (Claret and McIntosh, 1954), have been reported. The small number of such case reports, however, suggests that the risks of any type of neoplastic transformation in the ovary may not be increased by the existence of an intersex state.

On the other hand, the incidence of testicular tumours is undoubtedly increased in patients with an intersex state. Cryptorchidism is common in these cases, and the liability of ectopic testes to neoplasia is well documented (Dixon and Moore, 1953). It is significant that in these cases the tumour, although commonly a seminoma, is not infrequently a teratoma, embryonal carcinoma, teratocarcinoma or chorionepithelioma. For instance, in one series of 61 testicular tumours in male pseudohermaphrodites, there were 38 seminomas, 14 teratomas, and 9 miscellaneous tumours (Gilbert, 1942).

The remarkably close association between the gonadoblastoma and intersex states is now well documented. Of the cases described so far, all were associated with abnormal sex development, all proved to be chromatin negative, and in all the cases in which chromosome analysis is available an XY cell line was reported (Philip and Teter, 1964). It seems likely that the gonadoblastoma is a variant of the testicular seminoma rather than the ovarian disgerminoma, being a tumour of dysgenetic testes.

The age distribution of seminomas and disgerminomas is relevant to this argument. Testicular seminomas are relatively rare in younger age groups, only 12 per cent being found in males

under the age of 30 (Fergusson, 1962). By contrast, 80 per cent of ovarian dysgerminomas, in patients with normal sex development, occur before the age of 30 years (Santesson, 1947). However, many of the dysgerminomas which have been reported in intersex cases were found in older age groups, and the age distribution in these is more comparable to that of testicular seminomas rather than ovarian dysgerminomas (Willis, 1953). This may support the belief that these tumours in intersex cases are closely related to the seminoma type of tumour.

A simple concept of the relation of the dysgerminoma-seminoma type of tumour to intersex states emerge from this review. These tumours undoubtedly occur most commonly in the testes or ovaries of patients with normal sex development. However, the risk of developing a seminoma is increased in ectopic testes whether or not there is an associated intersex state. The incidence of other forms of neoplasms is also increased in ectopic testes, but probably to a lesser extent (Fergusson, 1962). This implies that the significant aetiological factor is possibly the site of the testis rather than the abnormal sex development. Furthermore, there is no evidence that the incidence of neoplasia is increased in the ovaries of intersex patients.

This argument has a clinical application. In intersex states there is an increased risk of gonadal neoplasia only if testicular tissue is present. This may be indicated by the presence of an XY cell line which is strong presumptive evidence of testicular tissue. However, it may occasionally arise in chromatin negative X monosomics with dysgenetic gonads. Furthermore, it is important to realize that the findings of a normal female karyotype does not exclude the presence of testicular tissue, for, as we have shown, a true hermaphrodite possessing both ovarian and testicular tissue may have an XX sex chromosome complement. As a corollary to this, the finding of a tumour of the seminoma-dysgerminoma type in an intersex patient is strong evidence for the presence of testicular tissue.

B. Arrhenoblastomas and Intersex States.

Pick (1905) reported the first description of an extremely well-differentiated arrhenoblastoma in a female patient. He remarked on its close resemblance to the typical tubular adenoma of the

undescended testis, and suggested that his case may in fact have been a testicular tumour arising from an ovotestis. However, Meyer (1931) later included Pick's tumour and similar cases as examples of well-differentiated arrhenoblastomas, and the status of the tubular adenoma of Pick as an ovarian tumour has been generally accepted. However, with the recognition of the testicular feminization syndrome (Morris, 1953), it is now appreciated that the presence of bilateral cryptorchidism is compatible with a female phenotype. Moreover, in this syndrome the undescended testes are commonly the site of tubular adenomas which may replace the whole testis. In these cases, if the opposite gonad is not examined, or if it is also replaced by the tumour, an erroneous diagnosis of ovarian arrhenoblastoma may be made. This risk is increased by the common finding of an ovarian-like stroma in the capsule of these testes (Morris and Mahesh, 1963).

It is impossible to know how frequently this error has been made. In two of our cases in which an arrhenoblastoma was initially reported, the correct diagnosis proved to be testicular tubular adenomas in genetic males. In an incomplete review of the literature, we have encountered nine cases reported as ovarian tumours in females where the true diagnosis may be testicular feminization (Behrend and Levine, 1936; Anderson, 1937; Dudman, 1939; Novak, 1943—2 cases; Jolles and Gleave, 1945; Goldberg and Maxwell, 1947; Javert and Finn, 1951; Langley, 1954). The features common to these cases are primary amenorrhoea, absence of masculinization, and an absent or rudimentary uterus.

Sex chromatin analysis will clarify the diagnosis in most of these cases. As we have shown, ovarian arrhenoblastomas are chromatin positive, while testicular tubular adenomas are chromatin negative. It should be emphasized that the histological appearance of a testicular tubular adenoma and a well-differentiated ovarian arrhenoblastoma may be indistinguishable. Not all of this type of tumour occur in male testes, and a number of cases have been described in parous women (Javert and Finn, 1951). This emphasises the value of routine sex chromatin analysis as an adjunct to histological examination in elucidating the nature of these tumours.

SUMMARY

1. A study of five cases of ovarian disgerminoma and five cases of arrhenoblastoma is reported.

2. All five patients with a disgerminoma were phenotypically normal females and had a normal female sex chromatin pattern, while three of them had a normal female karyotype. Of the five tumours originally reported as arrhenoblastomas, two proved to be testicular tubular adenomas replacing the undescended testes of genetic males with the testicular feminization syndrome.

3. A review is presented of 36 reported cases of intersex with tumours of the seminoma-disgerminoma type, with special reference to the nature of the ipsilateral and contralateral gonads, and the sex chromatin and chromosome analysis of the patient. It is concluded that no disgerminoma occurring in an intersex patient has been proven to have arisen in ovarian tissue, while in the majority of cases it has been shown to have originated in testicular tissue.

4. There is probably no increased risk of any type of neoplastic change in the ovaries of intersex patients. By contrast, there is an increased risk of testicular tumours, including the seminoma, in male intersexes. This is thought to be a function of the ectopic position of the testes rather than the abnormal sex development *per se*.

5. The histological similarity of the well-differentiated arrhenoblastoma and the testicular tubular adenoma may lead to errors in diagnosis. Sex chromatin analysis is invaluable in these cases.

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